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ORIGINAL ARTICLE

Sex-Specific Response of *Caenorhabditis elegans* to Methylmercury Toxicity

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Abstract

Methylmercury (MeHg), an abundant environmental pollutant, has long been known to adversely affect neurodevelopment in both animals and humans. Several reports from epidemiological studies, as well as experimental data indicate sex-specific susceptibility to this neurotoxicant; however, the molecular bases of this process are still not clear. In the present study, we used *Caenorhabditis elegans* (*C. elegans*), to investigate sex differences in response to MeHg toxicity during development. Worms at different developmental stage (L1, L4, and adult) were treated with MeHg for 1 h. Lethality assays revealed that male worms exhibited significantly higher resistance to MeHg than hermaphrodites, when at L4 stage or adults. However, the number of worms with degenerated neurons was unaffected by MeHg, both in males and hermaphrodites. Lower susceptibility of males was not related to changes in mercury (Hg) accumulation, which was analogous for both wild-type (wt) and male-rich *him-8* strain. Total glutathione (GSH) levels decreased upon MeHg in *him-8*, but not in wt. Moreover, the sex-dependent response of the cytoplasmic thioredoxin system was observed—males exhibited significantly higher expression of thioredoxin TRX-1, and thioredoxin reductase TRXR-1 expression was downregulated upon MeHg treatment only in hermaphrodites. These outcomes indicate that the redox status is an important contributor to sex-specific sensitivity to MeHg in *C. elegans*.

Keywords Methylmercury · Sex · Male · *C. elegans* · Antioxidant · Thioredoxin

Introduction

Methylmercury (MeHg) is an abundant environmental contaminant linked to many neurological defects. It has been reported to alter cognitive functions—memory and learning abilities—as well as neuromotor activity. The most adverse effects of MeHg exposure have been observed in newborn and young individuals, which are more susceptible to the toxicant (Antunes Dos Santos et al. 2016; Johansson et al. 2007; Prpic et al. 2017). Several mechanisms have been linked to MeHg neurotoxicity, including disruption of neurotransmitters and calcium homeostasis, as well as oxidative stress and impaired antioxidant protection (Farina and Aschner 2017; Farina et al. 2011).

In addition to neurodevelopmental effects, several epidemiological and experimental studies indicated on sex differences in MeHg susceptibility, with males commonly showing higher susceptibility to MeHg than females in neurobehavioral (Llop et al. 2013; Weston et al. 2014) or biochemical (Ruszkiewicz et al. 2016; Leung et al. 2018) evaluations. Given the rare incorporation of sex as a biological variable in MeHg studies, the mechanisms underlying sex differences have yet to be clearly understood.

In the present study, we used *Caenorhabditis elegans* (*C. elegans*), a recent model of choice in neurotoxicology, to address possible sex differences in MeHg susceptibility during development. *C. elegans* offers a unique tool to study developmental toxicity of MeHg and other neurotoxins, given the

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advantages associated with its genetic power and the ease of use (Ruszkiewicz et al. 2018; Avila et al. 2012). The model has been previously applied in several studies from our group (Helmcke and Aschner 2010; Martinez-Finley et al. 2013) and others (Wyatt et al. 2017; Rudgalvyte et al. 2017; McElwee et al. 2013) investigating mechanisms of MeHg toxicity; however, none of them considered sex differences. *C. elegans* has two sexes—hermaphrodites and males. Adult males differ from hermaphrodites in their morphology, anatomy, and expression of certain behaviors. Most sex-specific differences are established after hatching, over the course of larval development. Male larvae L1 display the same body shape as hermaphrodites, but from L2 stage, the shape changes as sexual organs begin to develop. The adult male can be distinguished from the hermaphrodite by its slimmer body, lack of vulva, clear ventral gonad, and distinctive tail, which bears a copulatory apparatus (Nguyen et al. 1999).

In this preliminary study, basic assays have been performed, addressing possible sex differences in worms' response to MeHg toxicity during development: lethality, dopaminergic (DAergic) neurodegeneration, mercury (Hg) accumulation and the status of key endogenous antioxidants—glutathione (GSH), and the cytoplasmic thioredoxin system, which consists of the thioredoxin (TRX-1) and thioredoxin reductase (TRXR-1).

Methods

C. elegans Strains and Maintenance

Strains used in this study were obtained from the Caenorhabditis Genetic Center (CGC; University of Minnesota): N2, wild-type (wt); CB1489M, *him-8* (e1489); BY200, *vtIs1* [*Pdat-1::GFP*] V; from Dr. Peter Swoboda lab: OE3265, *lin-15* (n765ts) X; *ofIs1* [*Ptrx-1::trx-1::GFP*; *pBHL98 (lin-15ab(+))*] IV; and from Dr. Simon Tuck lab: VB2619, *svEx889* [(*Ptrxr-1::trxr-1::GFP*) + (*Punc-122::RFP*)]. The *him-8* mutation causes X chromosome nondisjunction, yielding males to approximately (approx.) 37%. The *him-8* line is extensively studied in neuroscience and do not show any atypical phenotype (Hodgkin et al. 1979). For the other strains, males were generated through the heat shock method and maintained by crossing with hermaphrodites (Hodgkin 1983).

C. elegans strains were handled and maintained at 20 °C, grown on plates containing nematode growth medium (NGM) or 8P, and seeded with either *Escherichia coli* (*E. coli*) OP50 or NA22, respectively. Synchronous populations were obtained by isolating embryos from gravid worms using a bleaching solution (1% NaOCl and 0.25 M NaOH), and segregating eggs from debris by flotation on a sucrose gradient. After synchronizations, eggs were kept in M9 buffer (KH_2PO_4, Na_2HPO_4, NaCl) for 18–20 h. When most eggs hatched, these larvae L1 worms were washed three times with fresh M9, scored, and used for experiments or transferred to NGM plates with concentrated OP50, where they grow for additional 48 h to reach the L4 stage, or 96 h to reach adulthood.

MeHg Exposure

Synchronized worms at different developmental stage (L1, L4, or adult) were treated with methylmercury (II) chloride—MeHgCl (MeHg) for 1 h. Treatments involved combining worms (usually 2500 L1, 1000 L4, or 500 adults), 25 μl MeHg solution, and M9 buffer to a total volume of 500 μl. After exposure, worms were washed three times with M9 buffer and placed on OP50-containing NGM plates.

Lethality

Lethality tests were performed on synchronized worms at the developmental stage: L1, L4, and adult. The *him-8* and similarly treated wt animals were incubated for 1 h in M9 buffer with different concentrations of MeHg: 10–100 μM for L1 and adult and 40–300 μM for L4. Following treatment and washing, 20–60 worms (at least 20 males per group) were placed on OP50-seeded 35 mm NGM plates and scored, considering the sex of worms. Twenty-four hours later, only live worms were scored based on appearance and ability to move in response to poking with a platinum wire (L1 and L4) and pharyngeal pumping (adults). Due to the lack of visible morphological differences between sexes, the lethality of L1 worms was evaluated without distinguishing males and hermaphrodites. The L4 and adult males were distinguished from hermaphrodites by their morphological differences: slimmer body, clear ventral gonad, and a tail with copulatory apparatus. Each experiment was carried out independently eight times for each age. Results from each dose were calculated as % of control (0 μM MeHg). A sigmoidal dose-response model with a top constraint at 100% was used to draw the curves and determine lethal doses 50% (LD_{50}). Based on these studies all other experiments were carried out with MeHg doses of 20, 40 μM for L1 and adults; 40, 70, 140 μM for L4.

Mercury Level

Mercury (Hg) assay was performed on wt and *him-8*. Synchronized worms (20,000 L1, 2000 L4, or 2000 adult) were incubated for 1 h with MeHg (0, 20, 40 μM for L1/adults and 0, 40, 70, 140 μM for L4). Following treatment and washing, worms were placed on NGM plates with concentrated OP50 for 24 h. Twenty-four hours later, all worms (live and dead) were washed off the plate and washed with M9 three times (centrifuged 1 min, 500×*g*, 20 °C). Clean pellet was resuspended in 500 μl of ice cold 85 mM NaCl, flashed

freezed three times in liquid nitrogen, sonicated (3 × 20 s on ice), and centrifuged (10 min, 9000×*g*, 4 °C). The supernatant was collected, and a small aliquot (20 μl) was used for protein measurement while the rest of the sample was frozen (− 80 °C) and subsequently used for inductively coupled plasma-mass spectrometry (ICP-MS) analysis of Hg content. Four hundred microliters of the sample was digested in the microwave in the presence of 1.6 ml double distilled H_2O, 250 μl HNO_3 suprapur and 250 μl HCl suprapur. The analysis of the Hg content by ICP-MS was performed with the No gas mode. 0.01 μg/l rhodium was used as an internal standard, and the calibration was prepared in 10% HNO_3 suprapur and 10% HCl suprapur (concentration range 1–300 ng/l). Washing out solution used contained 1 ppm gold in 5% HNO_3 and 5% HCl (Lohren et al. 2015). Each experiment was repeated independently three times. The results were standardized to protein content and expressed as ng Hg/mg of protein.

Total Glutathione Level

Synchronized wt and *him-8* worms (40,000 L1, 10,000 L4, or 5000 adult) were incubated for 1 h with MeHg (0, 20, 40 μM for L1/adults and 0, 40, 70, 140 μM for L4). Following treatment and washing, worms were placed on NGM plates with concentrated OP50 for 24 h. Twenty-four hours later, sample preparation was performed as previously described (Caito and Aschner 2015). Briefly, all worms (live and dead) were harvested from the plate and washed with M9 three times (centrifuged 1 min, 500×*g*, 20 °C). The clean pellet was resuspended in 110 μl of extraction buffer and transfer to a chilled eppendorf tube, then vortexed (5 s), frozen with liquid nitrogen, and thawed in a water bath (37 °C); all steps repeated three times. Subsequently, sample was sonicated (20 s on ice), centrifuged (10 min, 9200×*g*, 4 °C), and the supernatant was collected to a new chilled tube and sample was frozen (− 80 °C) until analysis of glutathione (GSH) and protein content. Measurements of GSH was performed by the spectrophotometric method using 5,5′-dithio-bis (2-nitrobenzoic acid) (DTNB) with the presence of GSH standards (0.0625–40 μM) (Caito and Aschner 2015). Each experiment was repeated independently four times. Results were calculated based on protein content and expressed as nmol GSH/mg of protein.

For both GSH and Hg levels, the total *him-8* population was analyzed, which contains up to 40% males, because the number of nematodes required for these assays was not possible to reach by manually picking males and hermaphrodites.

Total Protein Level

Glutathione and Hg levels were standardized to total protein content, determined with the bicinchoninic acid (BCA) Protein Assay Kit (Thermo Fisher Scientific), according to the manufacturer's protocol.

Expression of the Cytoplasmic Thioredoxin and Thioredoxin Reductase

Transgenic lines expressing translational green fluorescent protein (GFP) reporters were used to evaluate the protein expression of TRX-1, *lin-15 (n765ts) X*; *ofIs1* [*Ptrx-1::trx-1::GFP*; *pBHL98 (lin-15ab(+))*] *IV*, and TRXR-1, *svEx889 [(Ptrxr-1::trxr-1::GFP) + (Punc-122::RFP)]*. Synchronized L4 worms were incubated for 1 h with MeHg (0, 70, 140 μM). Following treatment and washing, worms were placed on NGM plates with concentrated OP50. Twenty-four and 48 h following exposure worms (males and hermaphrodites) were manually picked and mounted on 4% agarose pads and anesthetized with 1 mM levamisole. Males were distinguished from hermaphrodites by their morphological differences: slimmer body, clear ventral gonad, and a tail with copulatory apparatus. The imaging settings were determined based on control worms. A PerkinElmer Spinning Disk Confocal microscope (× 60 magnification, scanning every 100 nm for XZ sections) was used for the imaging head neurons expressing TRX-1. GFP fluorescence intensity was measured in pixels inside neuron-surrounding equal-size sphere using Volocity software (PerkinElmer). Ten to 20 worms per group were analyzed from this strain. An epifluorescence microscope Nikon Eclipse 80i was used for imaging expression of TRXR-1 (× 4 magnification). Fluorescence intensity was measured in pixels from a square region of the anterior-most region of the intestine, behind the pharynx of animal, and analyzed using Image-J software. Twenty to 30 worms per group were analyzed from this strain. For each group, the data were normalized to control (0 μM MeHg) hermaphrodites and expressed as the mean ± SD of 4–5 independent trials.

Dopaminergic Neurodegeneration

Worms expressing GFP in dopaminergic (DAergic) head neurons: 4 cephalic (CEPs) and 2 anterior deirid (ADEs)—strain BY200 (*Pdat-1::GFP*)—were used to evaluate DAergic neurodegeneration after MeHg treatment. Synchronized L4 worms were treated as described above and placed onto concentrated OP50-seeded NGM plates for 48 h. Further, 25 worms (per sex) were mounted onto 4% agar pads and anesthetized with 1 mM levamisole. Males were distinguished from hermaphrodites by their morphological differences: slimmer body, clear ventral gonad, and a tail with copulatory apparatus. Worms were observed under an Olympus BX41 fluorescent microscope and scored for DAergic degeneration. Each worm was scored for absence (intact) or presence of any of the following morphological changes, representing degeneration: dendritic puncta formation, shrunken soma, loss of

dendrites, or loss of soma. Data were expressed as the percentage of worms with intact neurons. Each experiment was repeated independently four times. Representative confocal images of intact and degenerated neurons were taken using the PerkinElmer Spinning Disk Confocal Microscope.

Statistical Analysis

The statistical analyses of significance were performed using Prism 7 (GraphPad software) and SPSS 25 (IBM). A sigmoidal dose-response model with a top constraint at 100% was used to draw the curves and determine the LD_{50} in lethality analysis. Data were analyzed by multifactorial ANOVA followed by Tukey's multiple comparisons tests; to compare the mean values of various experimental groups, $P < 0.05$ was considered statistically significant. The results were expressed as mean ± SD.

Results

Males exhibited higher resistance to MeHg than hermaphrodites (Fig. 1b, c). At L4, *him-8* males exhibited lower susceptibility than *him-8* hermaphrodites ($P = 0.0033$, $F = 18.99$) (28% higher LD_{50}) and were more resistant than wt hermaphrodites to 100 and 140 μM of MeHg, although the response of both *him-8* male and hermaphrodites was not significantly different from wt hermaphrodites (Fig. 1b). Adult males were even more resistant to MeHg, with male *him-8* LD_{50} approximately twice higher than in *him-8* hermaphrodites ($P = 0.0006$, $F = 34.11$) and wt hermaphrodites ($P = 0.0007$, $F = 32.7$) (Fig. 1c). Notably, the susceptibility of L1 *him-8* worms to MeHg was significantly lower ($P = 0.0132$, $F = 10.85$) when compared to wt worms (Fig. 1a); however, the lethality of L1 *him-8* was evaluated without distinguishing males and hermaphrodites due to the lack of visible morphological differences between sexes. Overall, the higher resistance of L4 worms to MeHg matches previous observations (Helmcke et al. 2009). Herein, the percentage of males in the *him-8* strain accounted for 39% in the L4 and 42% in the adult stage (data not shown).

Neurodegeneration of dopaminergic (DAergic) neurons was unchanged upon MeHg treatment, both in males and hermaphrodites in the BY200 strain, although worms exhibited an insignificant tendency to decreased number of intact DA neurons at MeHg concentration close to LD_{50} (Fig. 2c). Similarly, lack of DAergic neurodegeneration was observed in the study where wt hermaphrodites (L4) were treated with MeHg for 15 h (Helmcke et al. 2009).

Mercury (Hg) levels found in worms collected 24 h after treatment indicate no difference between wt and *him-8* in Hg accumulation (Fig. 3). The strain effect was not significant ($P = 0.177$, $F = 1.884$); however, the dose effect was significant ($p < 0.0001$, $F = 31{,}656$)—with higher Hg levels in groups of worms exposed to higher MeHg doses—age effect ($P < 0.0001$, $F = 14.015$) and age × dose interaction ($P < 0.0001$, $F = 10.118$)—with the lowest levels of Hg found in L4 worms (Fig. 3b).

Total GSH levels in response to MeHg treatment did not change in wt worms but decreased significantly in *him-8* at L1 (38%, 40 vs. 0 μM MeHg) (Fig. 4a) and L4 (34%, 70 vs. 0 μM MeHg; 72%, 140 vs. 0 μM MeHg) (Fig. 4b) stage. In adult *him-8*, GSH levels remained unchanged (Fig. 4c). Multifactorial ANOVA revealed statistically significant strain effect ($P = 0.02$, $F = 10.647$), age effect ($P = 0.007$, $F = 5.33$), as well as age × strain interaction ($P < 0.0001$, $F = 10.590$)—with the highest GSH levels found in L1 wt worms—but in the absence of a dose effect ($P = 0.052$, $F = 2.502$). In the

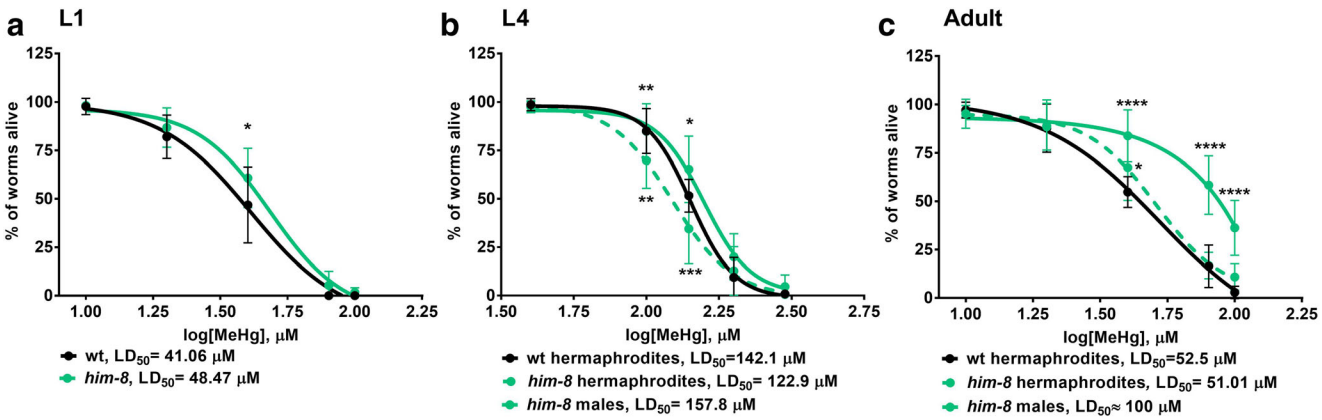

Fig. 1 Lethality of hermaphrodites vs. males. Synchronized wt and *him-8* worms at different developmental stage (L1, L4, and adult) were treated with MeHg for 1 h. Twenty-four hours later, alive worms were scored and based on the sigmoidal dose-response curves, lethal doses 50% (LD_{50}) were determined. At L1 stage, *him-8* strain was significantly more resistant to MeHg than wt ($P = 0.0132$, $F = 10.85$) (**a**). At L4 stage, *him-8* males exhibit lower susceptibility than *him-8* hermaphrodites ($P = 0.0033$, $F = 18.99$) (**b**), which was greater when worms were adult ($P = 0.0006$, $F = 34.11$); moreover, adult *him-8* males were significantly more resistant to MeHg than wt hermaphrodites ($P = 0.0007$, $F = 32.7$) (**c**) ($n = 8$). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$, $^{****}P < 0.0001$ vs. wt (post-hoc Tukey's test)

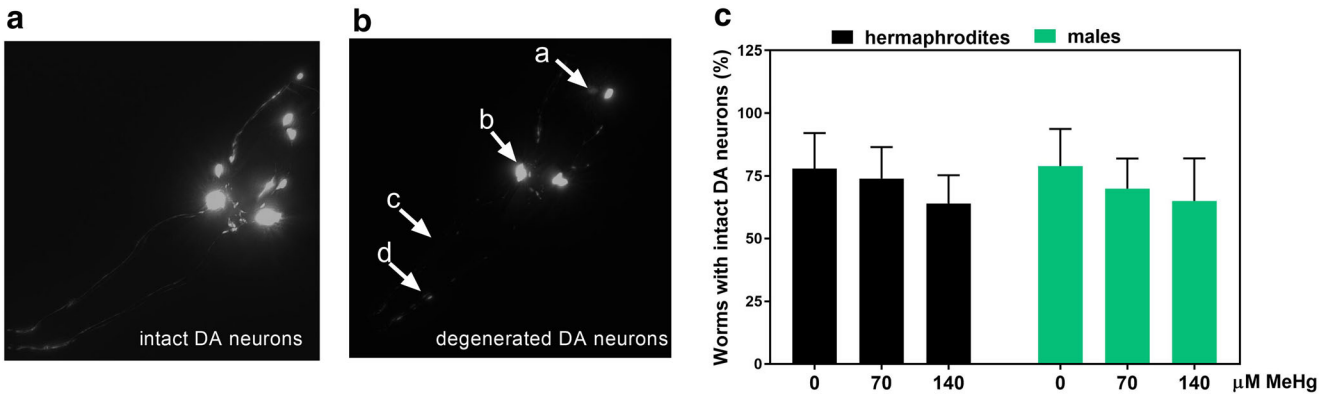

Fig. 2 Degeneration of DAergic neurons. *Pdat-1::GFP* (BY200) L4 worms were treated with MeHg for 1 h. Forty-eight hours later, alive worms (25 per group) were scored for degeneration of DAergic head neurons. Representative confocal images of intact (**a**) and degenerated (**b**) neurons, which exhibit loss of soma (a), shrunken soma (b), loss of dendrites (c), or dendritic puncta (d) are shown. The percentage of adult worms exhibiting degeneration of DAergic neurons was unaffected by MeHg treatment, both in males and hermaphrodites (**c**) ($n = 4$)

previous study, GSH levels remained unchanged upon MeHg treatment (0.5 h) when wt worms were at the L1 stage, but decreased in L4 worms exposed to MeHg for 15 h (Helmcke and Aschner 2010).

Cytoplasmic thioredoxin TRX-1 expression in ASJ neurons was significantly higher in males than hermaphrodites ($P < 0.0001$, $F = 58.044$) and was not significantly affected by MeHg exposure in both groups (Fig. 5a). Twenty-four hours post-treatment, the control males exhibited TRX-1 expression 103% higher than control hermaphrodites and 114% higher when groups treated with 140 µM MeHg were compared. Forty-eight hours post-treatment, the difference in TRX-1 expression between hermaphrodites and males were 88%, 74%, and 59% for groups treated with 0, 70, and 140 µM MeHg, respectively (Fig. 5a). Thioredoxin reductase (TRXR-1) expression was at a similar level in control males and hermaphrodites but decreased significantly upon 140 µM MeHg exposure in hermaphrodites only, 24 h (22%) and 48 h (23%) post-treatment (Fig. 5b). While the dose effect was significant ($P = 0.002$, $F = 7.578$), there was no significant sex effect ($P = 0.139$, $F = 2.293$). Both strains exhibited MeHg susceptibility similar to the wt animals, with comparable LD_{50} at the L4 stage (data not shown).

Discussion

Unlike in higher organisms, *C. elegans* males seem to be more resistant to MeHg toxicity when compared to hermaphrodites (Fig. 1). Epidemiological data (Llop et al. 2013; Marques et al. 2015; Grandjean et al. 1998; Sagiv et al. 2012) and various experimental studies (Ruszkiewicz et al. 2016; Edoff et al. 2017) suggest that males are affected by MeHg to a greater extent than females. However, the data are ambiguous, with

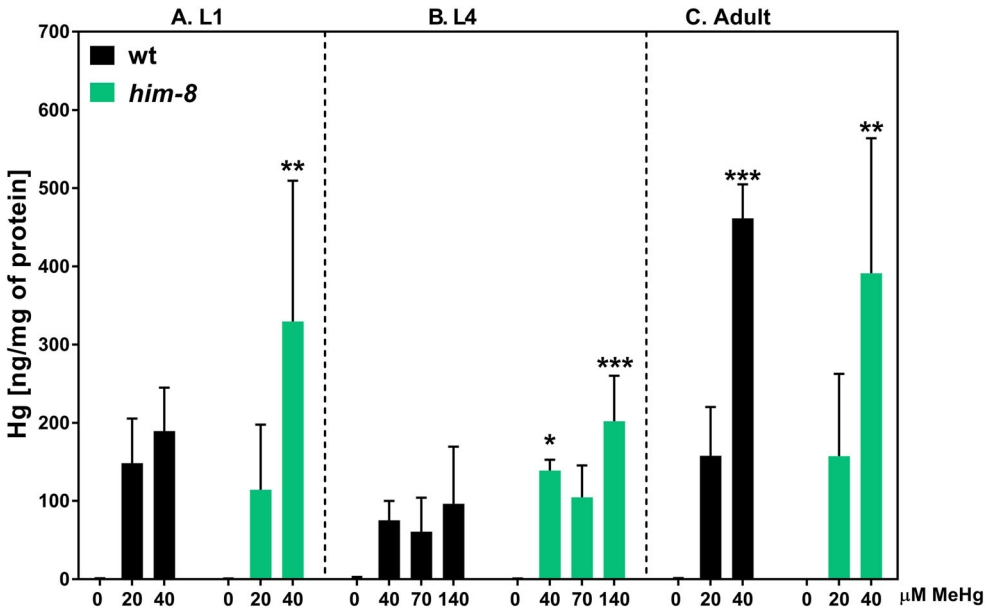

Fig. 3 Mercury (Hg) content. Synchronized wt and *him-8* worms at different developmental stage (L1, L4, and adult) were treated with MeHg for 1 h. Twenty-four hours later, worms were collected and extracts for Hg assay were prepared. Multifactorial AVOVA revealed significant dose ($P < 0.0001$, $F = 31,656$), age ($P < 0.0001$, $F = 14.015$) effects, and age × dose interaction ($P < 0.0001$, $F = 10.118$), but no significant strain effect ($P = 0.177$, $F = 1.884$), suggesting no difference between wt and *him-8* in Hg accumulation ($n = 3$). *$P < 0.05$, **$P < 0.01$, ***$P < 0.005$, vs. 0 µM MeHg (post-hoc Tukey's test)

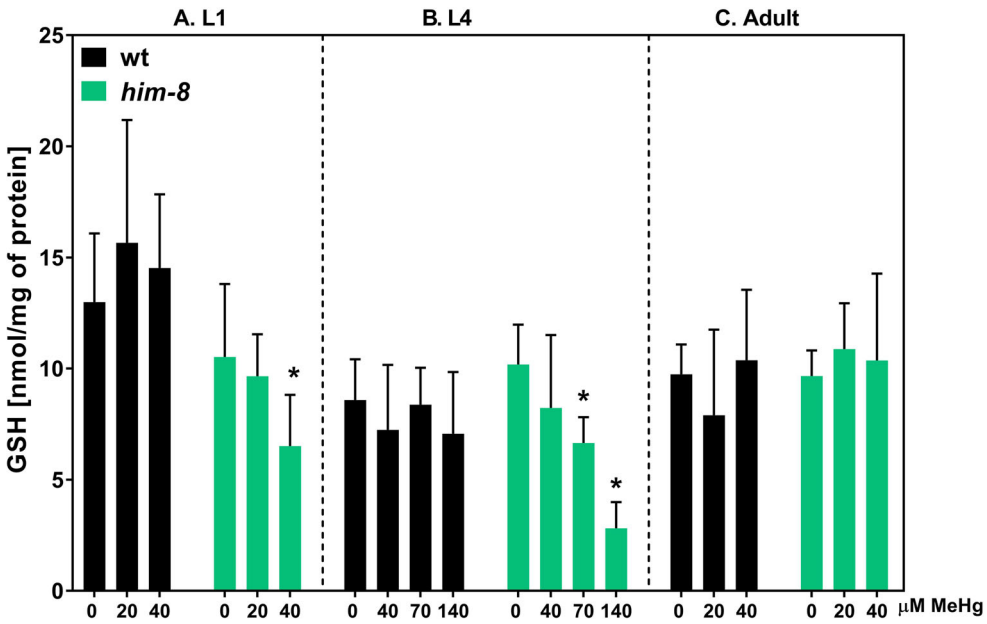

Fig. 4 Total glutathione (GSH) levels. Synchronized wt and *him-8* worms at different developmental stage (L1, L4, and adult) were treated with MeHg for 1 h. Twenty-four hours later, worms were collected and extracts for GSH assay were prepared. GSH levels in response to MeHg did not change in wt worms but decreased in *him-8* (L1 and L4) (**a**, **b**). ANOVA revealed significant age effect ($P = 0.007$, $F = 5.33$), strain effect ($P = 0.02$, $F = 10.647$), as well as age × strain interaction ($P < 0.0001$, $F = 10.590$) ($n = 4$). *$P < 0.05$ vs. 0 µM MeHg (post-hoc Tukey's test)

the results depending upon the experimental model or test used; thus, higher male susceptibility has yet to be confirmed (Llop et al. 2013; Goulet et al. 2003). Likewise, the mechanisms by which biological sex influences susceptibility of the developing brain to MeHg has yet to be addressed.

In *C. elegans*, the male resistance to MeHg was elevated during aging—adult males exhibited almost twice as high LD_{50} than hermaphrodites (Fig. 1c), whereas at the L4 stage, this difference was about 28% (Fig. 1b). Sex-specific changes in MeHg resistance were not accompanied by changes in neurodegeneration (Fig. 2). Age-dependent differences in lethality corresponded to levels of accumulated Hg content, with the highest resistance (Fig. 1b) being associated with the lowest Hg levels in L4 (Fig. 3b), both wt and *him-8*. This is probably a consequence of differential MeHg ingestion, due to worms' body changes during development—higher dose is required to penetrate the cuticle of the more developed worms, but in adult, open vulva during egg laying might provide an additional route of MeHg ingestion/uptake. These differences might also contribute to the sex-specific responses, since disparities between hermaphrodites and males in size or morphology (e.g., vulva) are changing during development and are particularly visible in adults, which simultaneously exhibit the biggest difference in susceptibility to MeHg.

Nonetheless, similar Hg levels 24 h post-treatment in the wt and *him-8* animals (Fig. 3) suggested that there is no significant difference in Hg accumulation between sexes. This means that regardless of morphological differences, MeHg exposure is probably at a similar level for males and hermaphrodites. We cannot exclude that the exposure was different, but during 24-h differential physiology resulted in analogous Hg levels in the two sexes. Sex-specific mechanism of uptake, metabolism, or excretion might affect the final Hg levels. Sex-dependent distribution and metabolism of mercurials were reported both in humans (Miettinen et al. 1971) and animals (Hirayama et al. 1987; Thomas et al. 1986), demonstrating more efficient excretion of the metal in females vs. males; however, to date, nothing is known about these differences in *C. elegans*.

Unique resistance to MeHg might be also due to differential molecular mechanisms of response and defense. In fact, male-enriched strain *him-8* exhibited a different pattern of response of GSH to MeHg. GSH levels in *him-8* were downregulated by MeHg at L1 and L4 stage, whereas they were unchanged in wt worms (Fig. 4). GSH levels remained constant in *him-8* adults, which exhibited the highest differences in susceptibility to MeHg (Fig. 1c), suggesting that the GSH status might contribute to sex-dependent resistance. On the other hand, the GSH content did not correspond fully to differences in lethality (wt vs. *him-8*), thus indicating that there must be an additional contributing factor to the MeHg resistance. It is also important to note, that we measured GSH and Hg levels in the total *him-8* population, which contains up to 40% males. Thus, we cannot exclude that sex differences have been masked by (still) majority of hermaphrodites. Although we believe that if males would exhibit significantly different levels of these molecules, this would be apparent.

More precise outcomes came from the TRX system evaluation, confirming the concurrence of changed antioxidant status and sex-dependent MeHg resistance. The expression of cytoplasmic thioredoxin TRX-1 was significantly higher in males than in hermaphrodites (Fig. 5a). Unlike in mammals, where TRX-1 is abundant, in *C. elegans* TRX-1 is exclusively expressed in ASJ sensory neurons, which has been linked to control of aging or dauer formation (Fierro-Gonzalez et al. 2011a; Fierro-Gonzalez et al. 2011b). Moreover, ASJ neurons

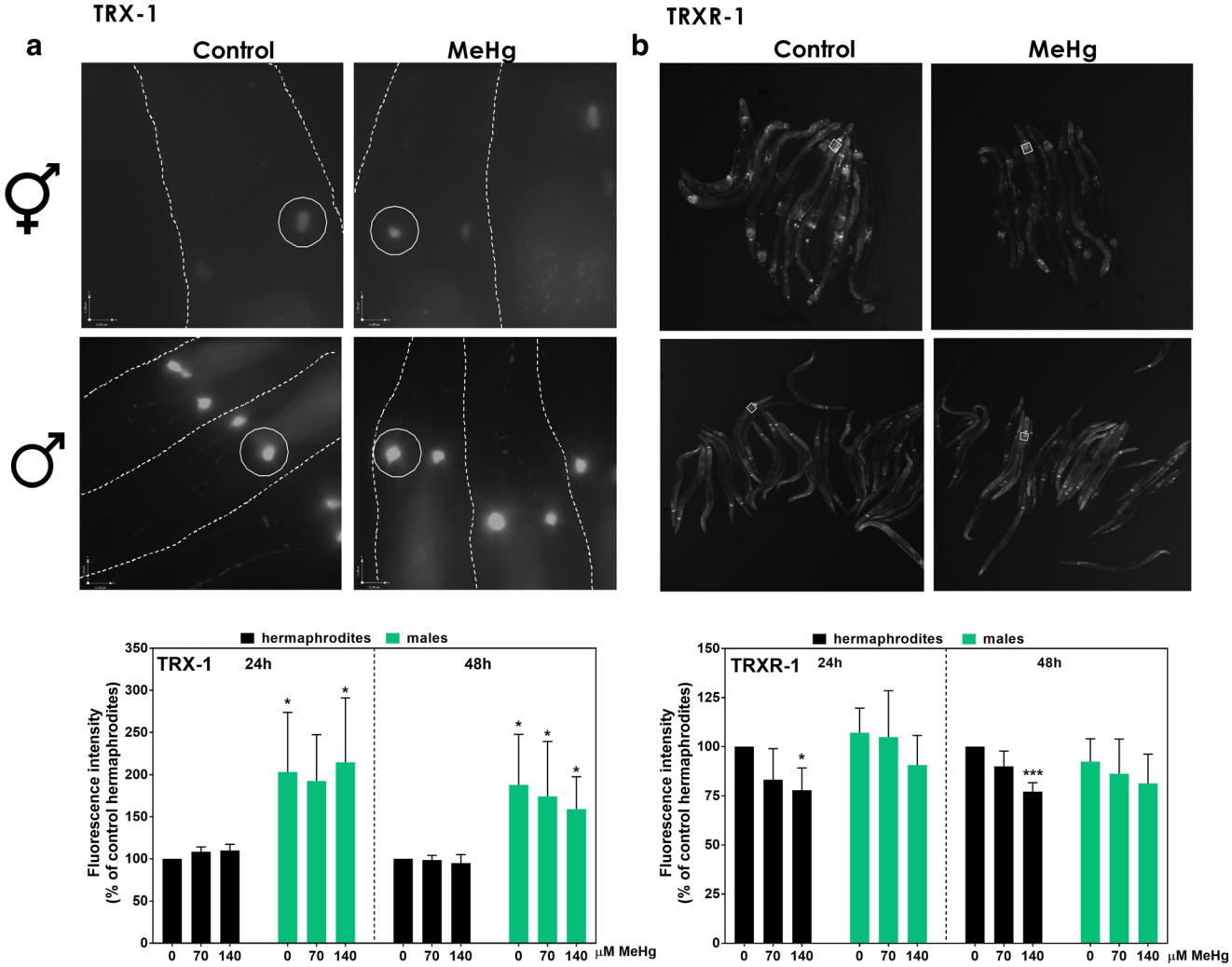

Fig. 5 The expression of the cytoplasmic thioredoxin system. Synchronized transgenic worms at L4 stage were treated with MeHg for 1 h. Twenty-four or 48 h later alive worms were manually picked for microscopic imaging. **a** 10–20 worms per group were analyzed for TRX-1 expression and GFP intensity was measured in pixels inside neuron-surrounding equal-size sphere. **b** 20–30 worms per group were analyzed for TRXR-1 expression and fluorescence intensity was measured in pixels from a square region of the anterior-most region of the intestine. Data were normalized to control hermaphrodites. TRX-1 expression in ASJ neurons was significantly higher in males (♂) than hermaphrodites ( ) ($P < 0.0001$, $F = 58.044$), and was not affected by MeHg (**a**). TRXR-1 expression was at a similar level in males and hermaphrodites, and MeHg downregulated TRXR-1 expression in hermaphrodites, but not in males (**b**) ($n = 4–5$). $^{*}P < 0.05$, $^{***}P < 0.001$ vs. 0 μM MeHg hermaphrodites (post-hoc Tukey's test)

are crucial in some male-specific behaviors (Hilbert and Kim 2017). TRX-1/TRXR-1 system in worms has been linked to protection against oxidative stress (Jee et al. 2005); thus, there is a possibility, that higher levels of TRX-1 might contribute to MeHg resistance through antioxidant protection. The response of TRXR-1 supports this hypothesis. Upon MeHg treatment, only hermaphrodites exhibited decreased TRXR-1 expression, whereas males preserved their level of the protein (Fig. 5b). This might lead to more efficient MeHg resistance in males and indicates on the cytoplasmic thioredoxin system (TRX-1/TRXR-1) as an important contributing factor. The sex-specific difference in response of the thioredoxin system was in agreement with our previous observations in mice, where greater TRX-1/TRXR-1 impairment in males corresponded to greater male susceptibility to MeHg (Ruszkiewicz et al. 2016). It is also noteworthy, that TRX and TRXR in mice brain were commonly higher in females than males (Saeed et al. 2009; Chen et al. 2010), which is opposite to worms; nevertheless, corroborating that elevated TRX/TRXR was associated with higher resistance to the toxicant.

In this study, for the first time, sex differences have been addressed in the response of *C. elegans* to MeHg toxicity, revealing some surprising outcomes. Unlike in mammals, in worms, the males exhibited higher resistance to MeHg. The sex-specific differences in susceptibility were not accompanied by changes in neurodegeneration and were not linked to differential Hg accumulation. The sex-dependent response

of antioxidant system—downregulated GSH levels, and differential expression of the cytoplasmic thioredoxin system TRX-1/TRXR-1 corresponded to male resistance, implicating on antioxidant status as an important contributor to the sex-specific susceptibility to MeHg.

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Compliance with Ethical Standards

Conflicts of Interest The authors declare that they have no conflict of interest.

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