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Biochemical and behavioral deficits in the lobster cockroach *Nauphoeta cinerea* model of methylmercury exposure

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Methylmercury (MeHg) is well-known for its neurodevelopmental effects both in animals and in humans. As an alternative to utilizing conventional animal models, this study evaluated behavioral and biochemical parameters using the nymphs of the lobster cockroach *Nauphoeta cinerea*. Animals were exposed to MeHg at 0, 0.03125, 0.0625, 0.125, 0.25 and 0.5 mg per g feed for 35 consecutive days. Locomotor activity and exploratory profiles were analyzed using video-tracking software during a 10 minute trial. Subsequently, biochemical estimations were carried out using cockroach heads. MeHg exposure caused behavioral impairment as evidenced by a significant decrease in distance travelled, time spent walking, turn angle and body rotation. The marked decrease in the exploratory profiles of MeHg-exposed cockroaches was confirmed by track plots, whereas occupancy plot analyses revealed a gradual dispersal in home-base formation, starting from 0.0625 mg per g feed. Biochemically, MeHg exposure significantly decreased acetylcholinesterase activity (AChE), an enzyme which plays a pivotal role in neurotransmission. Moreover, MeHg caused increased oxidative stress as evidenced by decreased total thiol levels and glutathione *S*-transferase (GST) activity, along with increased 2′,7′-dichlorofluorescein (DFCH) oxidation and thiobarbituric acid reactive substance (TBARS) production. In conclusion, these data demonstrated that *Nauphoeta cinerea* mimics the behavioral and biochemical deficits observed in rodents exposed to MeHg, thus highlighting its validity as an alternative model for basic toxicological studies.

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Introduction

Methylmercury (MeHg) is a long-established ubiquitous environmental contaminant whose toxicity is associated with neurological and developmental deficits in animals and humans.[1] Occupational exposure to inorganic mercury (Hg) can occur in industry, coal fired power plants and mining.[2,3] However, methylmercury produced as a result of methylation of inorganic mercury by methanorganic bacteria in an aquatic environment bio-accumulates in the aquatic food chain, eventually reaching the human diet.[1–4] Several epidemiological studies have shown that motor and cognitive impairments are the most common neurological alterations observed in MeHg-polluted populations.[5] Moreover, experimental points of evidence from rodent models indicated that developmental exposure to MeHg results in cognitive, motor and sensory impairment.[6–8]

The mechanisms involved in MeHg toxicity have been reported to include a decrease in endogenous antioxidant defense systems,[9,10] alteration of intracellular calcium homeostasis,[11] induction of oxidative stress,[12,13] modification of the presynaptic and postsynaptic glutamate status,[12,14] aberrant gene expression and epigenetic modifications.[15] The association between MeHg-induced motor deficits and cerebellar damage in rodents can be associated with impaired rotarod performance, motor coordination, open-field activity, retarded or abnormal walking ability, hind-limb dysfunction, delayed development of swimming ability, delayed spatial alternation, and radial arm-maze learning.[16,17] These behavioral patterns in rodents mimic symptoms observed in humans in their scope and affected systems.

Nevertheless, national and international government agencies have defined a need to reduce, refine, or substitute mammalian species in toxicological testing with alternative

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testing methods and non-mammalian models.[18] Indeed, zebrafish (*Danio rerio*), cockroaches (*Nauphoeta cinerea*, *Periplaneta americana* and *Phoetalia pallida*), fruit flies (*Drosophila melanogaster*), and nematode (*Caenorhabditis elegans*) have proven to be excellent non-mammalian models for neurotoxicology and neurological disease studies.[19–24] Cockroaches have been indicated as a potential non-mammalian model for assessing the toxicity and pharmacological effects of xenobiotics.[25–29] Besides the similarity in the biophysical principles of the nervous system functions in insects and mammals, cockroaches are smaller, easier to maintain and highly prolific animal models.[26,30]

The present study was designed to evaluate the behavioral and biochemical parameters in a non-mammalian species with special interest in cockroaches sub-chronically exposed to methylmercury. To achieve such a goal, we employed video-tracking software (ANY-maze, Stoelting, CO, USA) to assess the locomotor endpoints and exploratory profile of the experimental nymph cockroaches in both horizontal and vertical regions of a novel environment, which is a behavioral protocol used for assessing novelty-associated behavioral stress responses.[31] To our knowledge, this is the first study to describe the locomotory and biochemical changes in a *Nauphoeta cinerea* model of methylmercury exposure. Furthermore, acetylcholinesterase (AChE) activity, total thiol concentration, glutathione *S*-transferase (GST) activity, dichlorofluorescein (DCF) oxidation, and thiobarbituric acid reactive substance (TBARS) levels were determined in heads of MeHg-treated *Nauphoeta cinerea*.

Materials and methods

Chemicals

Methylmercury(II) chloride (MeHgCl), 1-chloro-2,4-dinitrobenzene (CDNB), glutathione (GSH), 2′,7′-dichlorofluorescein diacetate (DCFH-DA), thiobarbituric acid (TBA), acetylthiocholine iodide, and 5′,5′-dithiobis(2-nitrobenzoic acid) (DTNB) were procured from Sigma Aldrich (St. Louis, MO, USA).

Cockroach husbandry and treatments

The nymphs of *Nauphoeta cinerea* were used in this study because a developing organism is often more susceptible to toxic insult than the adult. Nymphs of the lobster cockroach *Nauphoeta cinerea* were obtained from the Laboratório de Bioquímica Toxicologica, Universidade Federal de Santa Maria, Brazil. They were reared in plastic boxes under standard conditions of a controlled temperature of 25 ± 1 °C and 70% relative humidity, and subjected to natural 12 h : 12 h light-to-dark photoperiod cycles. The insects had free access to water and standard cockroach food. One kilogram of the standard diet contained 500 g of corn meal, 350 g of wheat flour, 100 g of sugar (sugarcane sucrose), 5 g of commercial salt (NaCl supplemented with iodine, 20 μg of iodine g^{-1}), 25 g of casein and 20 g of powdered cow's milk. All the constituents of the feed were locally obtained from the supermarket. Methylmercury was added to the dry food as an ethanol solution and was left till the ethanol was completely evaporated. The control food was treated with an equivalent volume of ethanol. After ethanol evaporation, diets were stored at −20 °C. Experimental cockroaches were assigned to six groups consisting of 30 nymph cockroaches per group. The control group received only standard food whereas the remaining five groups were fed with food containing MeHg at 0.03125, 0.0625, 0.125, 0.25 and 0.5 mg per g feed for 35 consecutive days, corresponding to an ingestion of MeHg of approximately 1.01, 2.03, 4.24, 8.20 and 15.6 mg kg^{-1} per day, respectively based on calculations of food consumption undertaken during exposure. The exposure time and concentrations were chosen based on the preliminary range-finding experiments conducted to determine concentrations that would result in the survival of cockroaches long enough for the manifestation of neurobehavioral changes. All protocols and experiments performed in this study are represented in Fig. 1.

Behavioral experiments

Following MeHg exposure, the novel environment test was performed to evaluate the behavior pattern of cockroaches. Briefly, the cockroaches were randomly selected and placed in a white polystyrene box (15 cm in width × 15 cm in length × 7 cm in height) and their behavior was filmed during a 10 minute trial with a webcam (DNE webcam, Porto Alegre, Brazil) mounted above the open environment and connected to a laptop to record the videos. To ensure the same experimental conditions, all of the experiments were performed during the same period each day (from 10:00 a.m. to 4:00 p.m.). The behavioral parameters were automatically measured at a rate of 30 frames per second using a suitable video-tracking

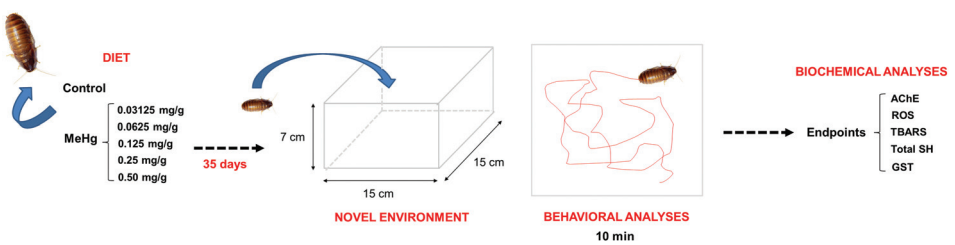

Fig. 1 Schematic design of the protocol, showing the experimental groups, period of treatment with dietary MeHg, as well as the behavioral test (novel environment task) and the biochemical endpoints assessed.

software (ANY-maze, Stoelting, CO, USA). Adequate care was taken when transferring the cockroaches from home containers to the novel environment to avoid handling stress. All the cockroaches were handled and tested using the standardized protocol (a similar manipulation, a time period of a day, and illumination) during the investigation.

Locomotor activity

The locomotor activity of the cockroaches was assessed using behavioral endpoints including the total distance travelled, immobility, body rotation, the number of falls, and turn angle (which represents the changes in the direction of the center point of the animal).

Vertical exploration

Cockroaches can exhibit a complex exploratory behavioral repertory that will depend on the novelty of the situation.[32] The vertical behavior of cockroaches in the new environment indicates its natural tendency to explore the novel situation. Here, the exploratory behavioral repertory was composed of both the horizontal movement (done in the bottom) and the vertical movement (climbing the walls of the new apparatus).[32,33] The vertical locomotion in cockroaches is complex and involves the coordinated movement and force generation by specific parts of the six legs of the cockroach.[33,34] Endpoints of vertical activity included the time spent and the number of entries into the periphery (the wall of the container) and bottom areas during the 10 minute trial.

Exploratory profile

An analysis of the exploratory profile of the cockroaches was performed using representative track and occupancy plots in order to represent the overall activity in both horizontal and vertical regions. The home base formation in the new environment during a trial was defined as a place in the arena for which the experimental animal showed a preference across time, both in terms of occupancy, and as the starting and ending points of exploratory tours.[35] The home base formation of cockroaches was assessed using behavioral data (basically transitions and time spent per section) and was confirmed by both track and occupancy plots.

Biochemical analysis

Following the behavioral testing, the cockroaches from control and MeHg-treated groups were anaesthetized in ice and weighed. Subsequently, the heads were carefully removed, weighed, homogenized in ice-cold 0.1 M phosphate buffer, pH 7.4, in the ratio of 1 : 40 (mg head : μL buffer) and centrifuged at 6000*g* for 10 min at 4 °C to obtain the supernatant, which was used for the biochemical estimations. The protein contents of head homogenates were determined by the Lowry method.[36]

Determination of the acetylcholinesterase activity

The determination of the acetylcholinesterase activity was carried out according to the method of Ellman *et al.*[37] The system consisted of 135 μL of distilled water, 20 μL of 100 mM potassium phosphate buffer (pH 7.4), 20 μL of 10 mM DTNB, 5 μL of sample, and 20 μL of 8 mM acetylthiocholine as a substrate. The degradation of acetylthiocholine iodide was measured for 5 min (30 second intervals) at 412 nm using a SpectraMax plate reader (Molecular Devices, CA, USA) and the results were expressed as μmol of thiocholine formed per min per mg protein.

Estimation of ROS production

The quantification of 2′,7′-dichlorofluorescein (DCFH) oxidation was performed to assess the intracellular level of ROS, a general index of oxidative stress.[38] The reaction mixture was made up of 150 μL of 0.1 M potassium phosphate buffer (pH 7.4), 40 μL of distilled water, 5 μL of DCFH-DA (200 μM, final concentration 5 μM), and 5 μL of the sample [1 : 100, weight (g of head)/(volume in mL)]. The fluorescence emission of DCF resulting from DCFH oxidation was monitored for 10 min (30 s intervals) at 488 and 525 nm excitation and emission wavelengths, respectively, using a SpectraMax plate reader (Molecular Devices, CA, USA). The rate of DCF formation was expressed as percentage of the control group.

Determination of the total thiol concentration

The total thiol content was determined according to the method previously described by Ellman.[39] The reaction mixture consisted of 170 μL of 0.1 M potassium phosphate buffer (pH 7.4), 20 μL of sample, and 10 μL of 10 mM DTNB. Following 30 minutes incubation at ambient temperature, the absorbance was measured at 412 nm. A standard curve was plotted for each measurement using GSH as a standard and the results were expressed as mmol of -SH per mg protein.

Determination of glutathione *S*-transferase activity

The glutathione *S*-transferase activity was determined according to the method of Habig and Jakoby[40] with slight modifications[41] using 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate. The assay reaction mixture consisted of 270 μL of a solution containing (20 mL of 0.25 M potassium phosphate buffer, pH 7.0, 10.5 mL of distilled water, and 500 μL of 0.1 M GSH at 25 °C), 20 μL of sample (1 : 50; w/v dilution, *i.e.*, 1 g of head : 50 mL of buffer), and 10 μL of 25 mM CDNB. The reaction was monitored for 5 min (30 second intervals) at 340 nm in a SpectraMax plate reader (Molecular Devices, CA, USA) and the data were expressed as μmol min^{-1} per mg protein using a molar extinction coefficient (ε) of 9.6 mM^{-1} cm^{-1} for the CDNB conjugate.

Thiobarbituric acid reactive substance determination

Lipid peroxidation end products were determined as thiobarbituric acid reactive substances (TBARS) as reported earlier.[42] Briefly, tissue samples were obtained by homogenizing the heads of cockroaches in chilled 0.1 M potassium phosphate buffer (pH 7.4) in the ratio of 1 : 5 (mg head : μL buffer). The stock reagent contained equal volumes of trichloroacetic acid (10%, w/v) and 2-thiobarbituric acid (0.75%, w/v) in 0.1 M HCl. One volume (100 μL) of the tissue supernatant and two volumes (200 μL) of the stock reagent were incubated at 95 °C for 60 minutes. After a cooling period, they were centrifuged at

8000*g* for 10 minutes and the absorbance of the supernatant was measured at 532 nm. The TBARS values were normalized by the cockroach head weight. TBARS tissue levels were expressed as nmol MDA per g tissue.

Statistical analyses

The data were expressed as the mean ± SD. The normal distribution and homogeneity of the data were confirmed by Kolmogorov–Smirnov and Bartlett's tests, respectively. Further statistical analyses were carried out by ANOVA followed by the Newman–Keuls multiple comparison test. Statistical significance was considered when $p < 0.05$.

Results

Locomotor activity

The general locomotor activities of control and MeHg-exposed cockroaches within the apparatus during a 10 minute trial in the novel environment are presented in Fig. 2. The endpoint analyses showed that cockroaches exposed to MeHg showed a dose-dependent significant decrease in the total distance travelled, turn angle and body rotation when compared with the control ($p < 0.05$). Also, the turn angle which is indicative of the turning behavior of MeHg-exposed cockroaches decreased significantly in comparison with the control group. However, the number of falls, the time of immobility and recurrent episodes of immobility of cockroaches exposed to MeHg were significantly increased during the trial. Thus, MeHg caused significant dose-dependent adverse effects on the locomotor activity when compared with the control (Fig. 2).

Vertical exploration

The vertical exploratory behaviors of control and MeHg-exposed cockroaches are presented in Fig. 3. Endpoint analyses revealed that MeHg exposure significantly decreased the time spent in the periphery, the mean visit and the number of transitions to the periphery when compared with the control ($p < 0.05$). However, the total time spent and the average time spent per visit in the bottom area were significantly increased when compared with the control. In addition, cockroaches exposed to MeHg showed increased periods of immobility in the bottom area when compared with the control.

Exploratory profile

The exploratory profiles of the control and MeHg-exposed cockroaches were assessed using both horizontal and vertical dimensions of the novel environment. The representative track

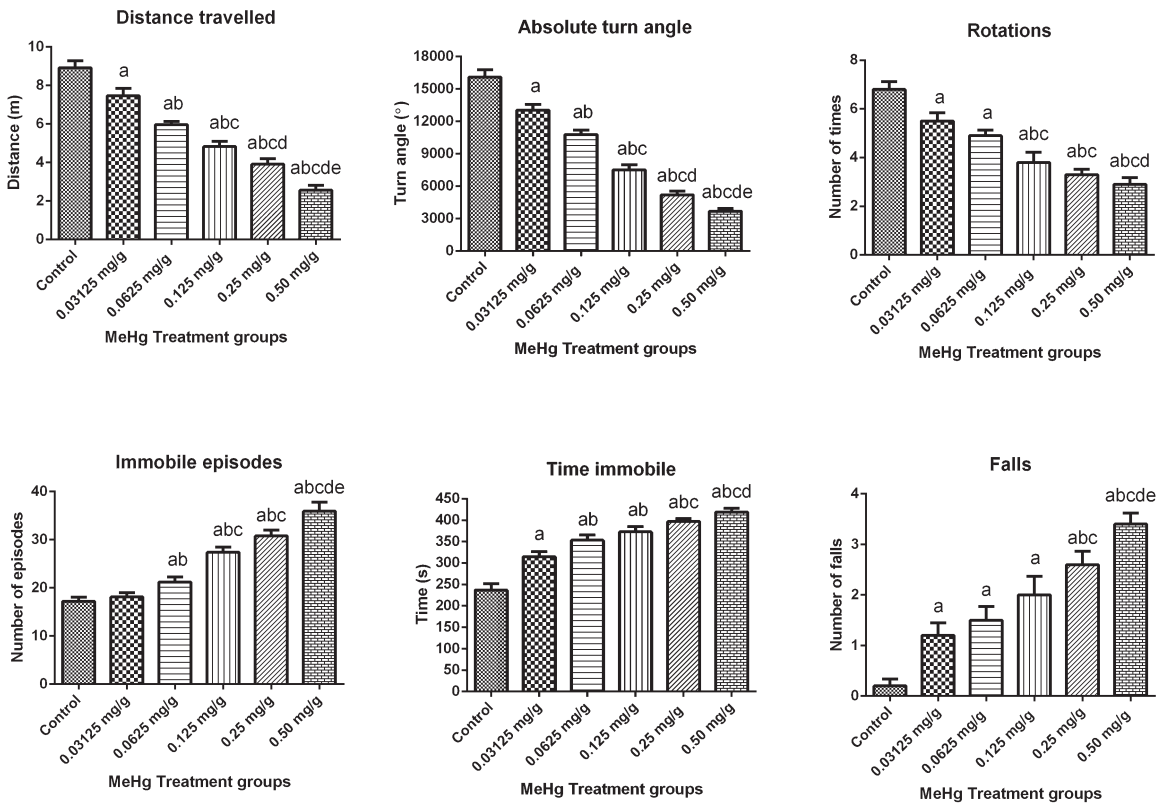

Fig. 2 Locomotion and motor endpoints evaluated in control and methylmercury (MeHg)-exposed cockroaches. The figure depicts the effects of MeHg on endpoints including the total distance travelled, absolute turn angle, the number of body rotations, immobile episodes, time immobile, and the number of falls during a 10 min trial. The data are expressed as mean ± S.D. for 28 cockroaches per group. a: Values differ significantly from the control ($p < 0.05$). b: Values differ significantly from 0.03125 mg per g feed ($p < 0.05$). c: Values differ significantly from 0.0625 mg per g feed ($p < 0.05$). d: Values differ significantly from 0.125 mg per g feed ($p < 0.05$). e: Values differ significantly from 0.25 mg per g feed ($p < 0.05$).

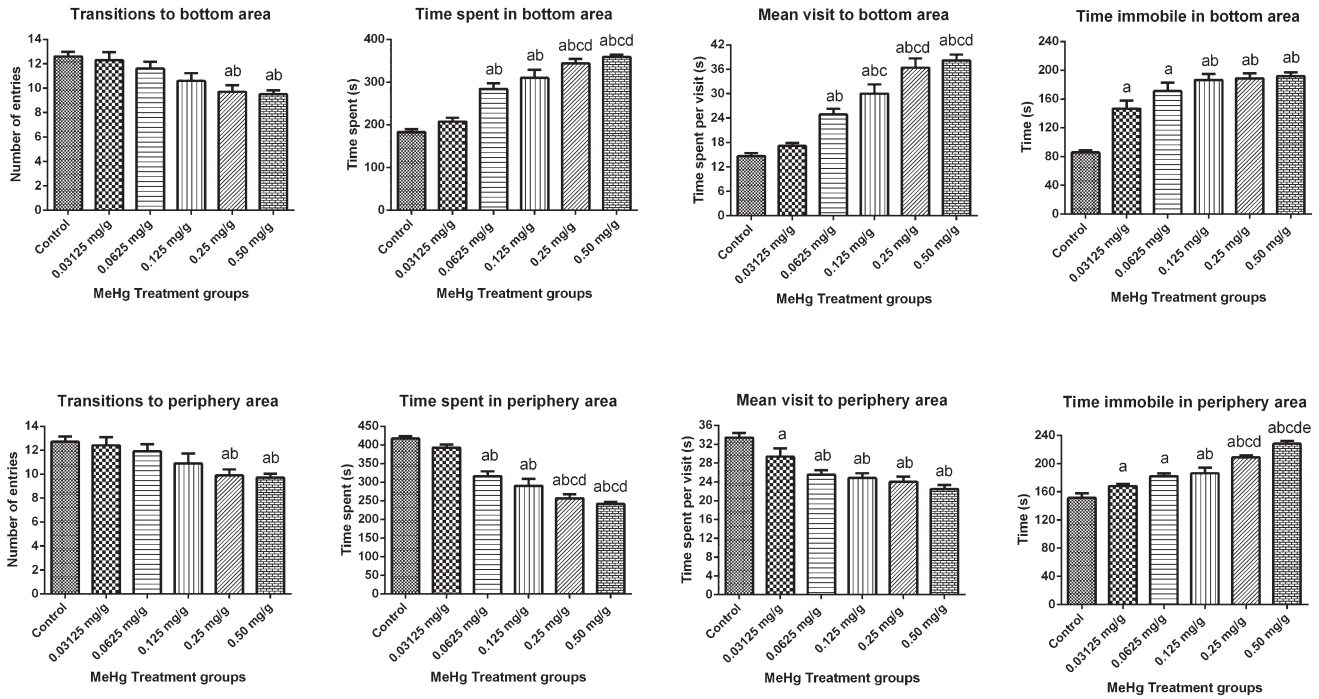

Fig. 3 Effect of MeHg exposure on the vertical activity of cockroaches, showing the number of entries, time spent, average duration of entry, and time immobile in both the bottom and the periphery of the novel environment during a 10 min trial. The data are expressed as mean ± S.D. for 28 cockroaches per group. a: Values differ significantly from the control ($p < 0.05$). b: Values differ significantly from 0.03125 mg per g feed ($p < 0.05$). c: Values differ significantly from 0.0625 mg per g feed ($p < 0.05$). d: Values differ significantly from 0.125 mg per g feed ($p < 0.05$). e: Values differ significantly from 0.25 mg per g feed ($p < 0.05$).

plots of the walking traces of individual cockroaches within the apparatus are presented in Fig. 4A. Dietary exposure to MeHg caused an obvious decrease in the exploratory activity of the treated cockroaches. The control cockroaches showed a normal behavioral profile by exploring both the periphery and bottom areas of the apparatus. Conversely, track plots showed the locomotor impairment in the MeHg-exposed cockroaches considering the exploration in the periphery of the apparatus. Moreover, the representative occupancy plots of control and MeHg-exposed cockroaches are presented in Fig. 4B. The occupancy plots of MeHg-exposed cockroaches were characterized by decreased exploration with a concomitant increase in the time spent in some specific regions of the arena. Specifically, the control and cockroaches fed with MeHg at 0.03125 mg per g feed showed a distinct exploration, with a regular return to the same location. However, cockroaches exposed to MeHg at 0.0625 mg per g feed and above returned to different points of the apparatus during the trial.

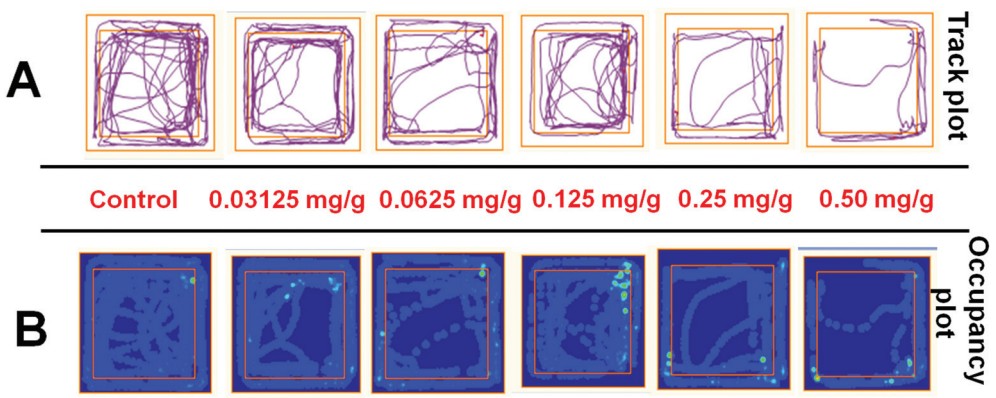

Fig. 4 Overall exploratory profiles of control and MeHg-exposed cockroaches represented by track and occupancy plots during a 10 min trial. (A) The representative track plots showing the path traveled by cockroaches in the apparatus. (B) The representative occupancy plot of exploratory activity in the open-field. The light green spots in the occupancy plot indicate home base formation, the regions of frequent immobile episodes. The data were analyzed using video-tracking software (ANY-maze, Stoelting Co., USA).

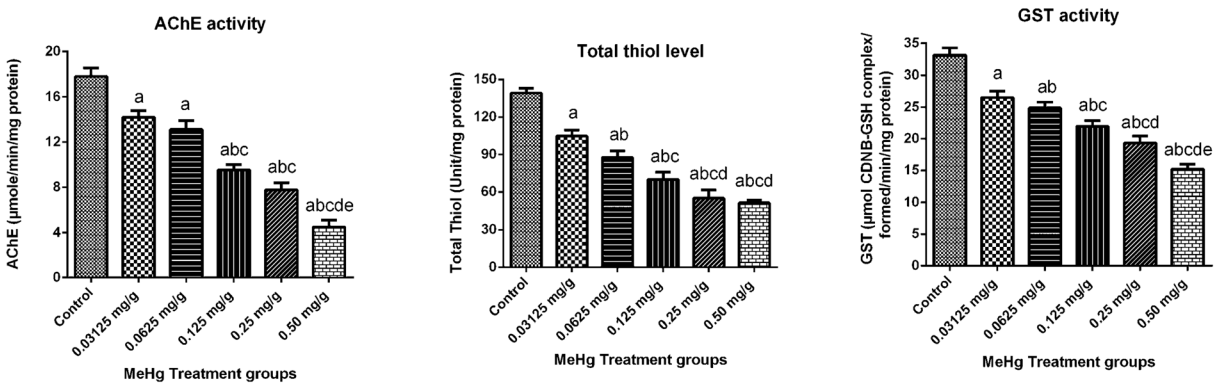

Fig. 5 Biochemical endpoints depicting the acetylcholinesterase activity, total thiol level, and glutathione *S*-transferase activity in heads of control and MeHg-exposed cockroaches. The data are expressed as mean ± S.D. for 28 cockroaches per group. a: Values differ significantly from the control ($p < 0.05$). b: Values differ significantly from 0.03125 mg per g feed ($p < 0.05$). c: Values differ significantly from 0.0625 mg per g feed ($p < 0.05$). d: Values differ significantly from 0.125 mg per g feed ($p < 0.05$). e: Values differ significantly from 0.25 mg per g feed ($p < 0.05$).

Acetylcholinesterase activity and oxidative stress parameters

Fig. 5 and 6 show the activity of acetylcholinesterase and oxidative stress indices in heads of control and cockroaches exposed to MeHg for 35 consecutive days. Sub-chronic exposure to MeHg at all the investigated doses caused a significant ($p < 0.05$) decrease in acetylcholinesterase and GST activities as well as in the total thiol concentration in the treated cockroaches. MeHg exposure significantly decreased acetylcholinesterase activity by 20.3%, 26.4%, 46.6%, 56.5% and 74.8% whereas GST activity was decreased by 23.1%, 28%, 36.8%, 44.7% and 57.2% at 0.03125, 0.0625, 0.125, 0.25 and 0.5 mg of MeHg per g of diet respectively, when compared with the control group. Moreover, MeHg exposure decreased the total thiol concentration by 24.8%, 37.1%, 49.7%, 60.3% and 63% at 0.03125, 0.0625, 0.125, 0.25 and 0.5 mg per g feed respectively, when compared with the control group. As revealed by DCF fluorescence intensity, the levels of ROS generation in the heads of MeHg-exposed cockroaches increased significantly in comparison with the control. Similarly, MeHg exposure significantly elevated the levels of TBARS in the treated cockroaches. The increases in the ROS level were 77%, 88.6%, 92.8%, 94.7% and 104.2% whereas the TBARS level increased by 113.5%, 258.5%, 360.1%, 365.6% and 407.9% at 0.03125, 0.0625, 0.125, 0.25 and 0.5 mg per g feed respectively, when compared with the control group.

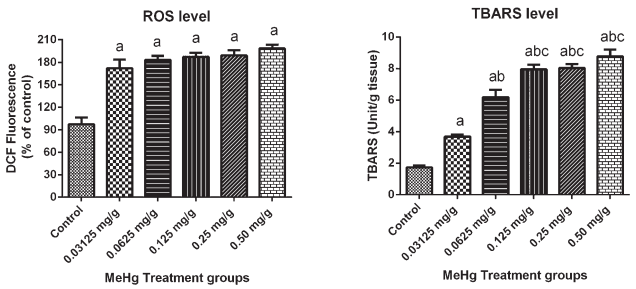

Fig. 6 Oxidative stress biomarkers depicting the reactive oxygen species and thiobarbituric acid reactive substance production in heads of control and MeHg-exposed cockroaches. The data are expressed as mean ± S.D. for 28 cockroaches per group. a: Values differ significantly from the control ($p < 0.05$). b: Values differ significantly from 0.03125 mg per g feed ($p < 0.05$). c: Values differ significantly from 0.0625 mg per g feed ($p < 0.05$).

Discussion

The use of alternative models in neuroscience to assess both the safety and toxic effects of chemical substances to the brain has been widely encouraged.[18,43] Alternative testing methods and non-mammalian models designed to study neurotoxicity of xenobiotics need to provide information about the output of the nervous system which often manifests in the behavior. In this regard, this is the first study that reports behavioral tests to evaluate locomotor parameters, motor coordination and exploratory behavior as well as biochemical endpoints in *Nauphoeta cinerea* following exposure to an established neurotoxicant methylmercury.

Endpoint analyses in the present study showed that MeHg exposure resulted in significant impairment in locomotor parameters of the cockroaches as evidenced by a decrease in the total distance travelled along with the increase in the number of falls, time immobile and recurrent episodes of immobility. Moreover, body rotation and turn angle may be considered as important locomotor parameters associated with motor coordination during bodily movements.[44] In the present investigation, MeHg-exposed cockroaches showed decreased body rotation and turn angle, indicating alteration in the motor posture patterns. In agreement with the previous studies from both human outbreak cases of intoxications and rodent models,[45] MeHg exposure adversely affected the locomotion of the experimental cockroaches.

The exploration in the periphery and bottom areas of the novel apparatus was determined to verify the preference of cockroaches for each area. Naturally, control cockroaches entered the periphery area of the novel environment and subsequently returned to the bottom of the apparatus. The

exploratory behavior of cockroaches is complex and depends on factors such as novelty of the situation.[32] The adverse effects of MeHg exposure on the exploratory activity of the cockroaches were pronounced. The track and occupancy plots revealed a decrease in the exploration during the trial. Exploration of the vertical areas is an effective strategy to escape or avoid predation, a response similar to the phenomenon of thigmotaxis (attraction to the wall) observed in rodents and fish in a novel environment.[46,47] Besides, the cockroach gains from complete exploration of the environment when searching for food. In general, our data showed that control cockroaches prefer to spend more time in the periphery than the bottom area, indicating the preference for establishing probably a defensive behavior. Moreover, control cockroaches seem to display a home base formation, which is considered a safe place where an animal spends more time in and repeatedly returns to after exploring the environment. However, dietary MeHg exposure induced a significant disruption in this behavior, as confirmed by occupancy plots. Although the significance of this phenomenon still requires further investigation. The changes in exploratory profiles of the cockroaches could be attributed to impairment in the locomotion following MeHg exposure. These behavioral alterations negatively impact the insect orientation and locomotion and may pose serious ecological consequences. To our knowledge, this is the first study to investigate MeHg-induced alteration in the explorative activity of cockroaches in a novel environment.

Mechanistically, it is well known that the neurotransmitter acetylcholine participates in the regulation of motor function, locomotion, and exploration.[48] Acetylcholinesterase hydrolyses acetylcholine at synapses, thus playing a crucial role in cholinergic neurotransmission. Moreover, AChE can be considered a biomarker for evaluating the function of the nervous system and its activity is widely used to diagnose neurodegeneration diseases and defects.[49,50] The decrease in the acetylcholinesterase activity observed in the present study could prevent the normal neurotransmission and may thus be related to the decrease in the motor function, locomotion, and exploration seen in the MeHg-exposed cockroaches. Our finding is in accordance with previous studies which showed that MeHg exposure resulted in decreased acetylcholinesterase activity.[51,52] However, MeHg is not expected to react directly with AChE; thus the exact mechanism of inhibition of MeHg on AChE activity warrants further investigation.

MeHg-induced neurotoxicity has been linked to disrupted antioxidant homeostasis in rodents as well as in *in vitro* studies.[53] The glutathione antioxidant system has been reported to be a possible molecular target for MeHg toxicity in the developing brain.[8] Moreover, GST is an important detoxification enzyme involved in the conjugation of GSH with MeHg.[54] In the present investigation, the heads of cockroaches exposed to MeHg showed a marked decrease in the GST activity and total thiol concentration. Assessment of total thiol concentration is an established indirect oxidative stress biomarker to determine chemical changes in the thiol groups of proteins and peptides of various lengths.[55] The decrease in GST activity and total thiol level in MeHg-exposed cockroaches, which is also in agreement with previous observations,[56,57] may indicate an impairment in the excretion of MeHg and a state of cell metabolism more prone to oxidative stress in the cockroaches.

The molecular mechanisms underlying the interference of MeHg with GST have not been studied in detail. One of the difficulties in the detailed study of interaction of mercurials with thiol-containing proteins and enzymes is the extremely high affinity of mercurial for thiols and selenols groups.[58] For GST isoforms, the situation is even more complex and difficult to be solved experimentally, because inorganic and organic mercury can also react with the GST substrate, *i.e.*, GSH.[59] Despite the technical difficulties, Dierickx[59] demonstrated that both organic and inorganic mercury compounds interacted with rat hepatic GST isoforms by direct binding to these proteins. Although the mechanism of cockroach GST inhibition by MeHg was not investigated here, we can speculate that part of the inhibitory mechanism may be related to a direct interaction of MeHg with GST, particularly those containing the –SH group.[60,61] Furthermore, MeHg may modulate the expression of GST by interfering with electrophile sensitive transcription factors, as reported for worms.[62]

Lipid peroxidation is an oxidative hallmark of methylmercury-induced neurotoxicity in rodents.[13,63] MeHg-exposed cockroaches showed elevated ROS and TBARS levels, thus confirming an increased lipid peroxidation in the present study. In contrast to the results on behavioral skills, as well as on the AChE activity, thiol levels and GST activity, the data on ROS were not dose-dependent. The reason for this is not presently understood, but may indicate that subtle changes in different parameters involved in the maintenance of cellular redox state (for instance, GST and thiol groups) can result in disproportional production of ROS. Overproduction of ROS subsequently overwhelmed the antioxidant defense system in the head of the cockroaches, resulting in lipid peroxidation in the exposed insects.

Taken together, the findings from the present study corroborate with the results obtained from rodents and humans that MeHg exposure poses a serious behavioral and biochemical toxicity risk to developing organisms. Thus, this study evidenced the use of *Nauphoeta cinerea* as a valid alternative model organism for basic toxicological studies which may offer new insights for translational neuroscience research before the conventional vertebrate testing.

Conflict of interest

The authors have no conflicts of interest to declare.

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