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Research report

Aging, motor function, and sensitivity to calcium channel blockers: An investigation using chronic methylmercury exposure

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HIGHLIGHTS

- Two age groups of mice were chronically exposed to MeHg and nimodipine for 8.5 months.
- MeHg produced age-independent effects on wheel-running and rotarod performance.
- MeHg run decreased distance and speed of wheel-running but not the motivation to run.
- Nimodipine provided age-dependent protection from MeHg insult.
- Event analysis detected reliable indicators of MeHg toxicity.

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ABSTRACT

Methylmercury (MeHg) neurotoxicity is thought to be mediated, in part, by dysregulation of calcium (Ca^{2+}) homeostasis, a mechanism that may also slowly and progressively degrade neuronal function during normal aging. Longitudinal studies of MeHg exposure provide a powerful approach to studying neural and behavioral mechanisms by which both MeHg toxicity and aging affect motor function. Wheel-running and rotarod performance were assessed in two age groups of BALB/c mice chronically exposed to 0 or 1.2 mg/kg/day MeHg and 0 or 20 mg/kg/day nimodipine, a 1,4-dihyrdopyridine L-type calcium channel blocker (CCB), for approximately 8.5 months. Adults began exposure on postnatal day (PND) 72 and retired breeders on PND 296. A log-survivor bout analysis partitioned wheel-running into bouts that identified motor (within-bout rates) and motivational (bout-initiation rates) influences. Retired breeders ran farther, because of a higher bout-initiation rates, but performed more poorly on the rotarod than younger adults, a difference unaffected by nimodipine. MeHg produced relatively age-independent deficits in wheel-running and rotarod performance, whereas nimodipine afforded greater protection to adult mice than to retired breeders. Rotarod performance and within-bout response rate were more sensitive to and more reliable predictors of MeHg toxicity than bout-initiation rate, which was least affected by MeHg exposure. Thus the motivation to run was unimpaired as the ability to do so declined. While chronic MeHg exposure produced functionally similar behavior deficits between age groups, the age-dependent neuroprotection by nimodipine supports the notion that underlying neurobiological systems mediated by Ca^{2+} signaling, are differentially affected in older adults.

1. Introduction

Chronic adult-onset exposure to the global pollutant methylmercury (MeHg) has been linked to nervous system damage, "glove and stocking" sensory disturbances, weakness, cerebellar ataxia, and visual and auditory dysfunction in human

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populations [1–4]. Lower exposure produces mild sensorimotor dysfunction [5–7] and tremor [8–10]. Accordingly, sensorimotor dysfunction is a primary behavioral marker of protracted adult-onset MeHg exposure. Unknown exposures to other contaminants in epidemiological studies and potential comorbidity with other diseases or differential sensitivities that may arise throughout the age span complicate the interpretation of these findings. While there is good evidence that MeHg can accelerate aging in sensory and motor domains [11–17], there is paucity of literature describing differential consequences of early and late adult-onset MeHg exposure explicitly

In rodent models, postnatal MeHg exposure produces sensorimotor dysfunction reminiscent of that seen in humans, including impaired gait and balance, reduced open-field activity, locomotion and wheel-running, diminished somatosensory function, and hind-limb flexion [18–24]. More recently, Hoffman and Newland [25] reported that chronic MeHg exposure (2.6 mg/kg/day MeHg) disrupted wheel-running and rotarod performance in adult mice and Shen et al. [26] reported that chronic MeHg exposure (1.2 mg/kg/day MeHg) produced deficits in high-rate nose-poking. Both studies used bout analysis models to reveal the microstructure of behavior, which yielded measures of motor function in the maximum rate of running or nose-poking and motivation in the rate at which bouts of running or nose-poking were initiated. In both studies, MeHg impaired motor function as measured by a reduction in within-bout response rate, but both running and nose-poking continued in the face of impairment, indicating that the motivation to engage in these types of behavior could be decoupled from the ability to do so using a bout analysis.

Both MeHg and aging act by increasing Ca^{2+} influx into the nerve terminal or otherwise disrupting intracellular Ca^{2+} homeostasis, observations that support a hypothesis that preventing excessive increases in intracellular Ca^{2+} will protect against MeHg neurotoxicity or age-related deterioration. Nimodipine, a 1,4-dihyrdopyridine L-type CCB reduces Ca^{2+} influx into the nerve terminal, has excellent selectivity for the CNS and shields neurons against CNS insult [27]. *In vitro* [28–30] and recent *in vivo* studies [25,26,31,32] have shown that nimodipine and similar CCBs protect against MeHg toxicity. For example, in Hoffman and Newland's [25] study, chronic dietary nimodipine (2 or 20 mg/kg/day) dose-dependently blocked MeHg-induced deficits (2.6 mg/kg/day), with 20 mg/kg/day wholly blocking MeHg-induced deficits MeHg-induced deficits (2.6 mg/kg/day). Using a high-rate nose-poking task, Shen et al. [26] extended these findings by showing that nimodipine neuroprotection (20 mg/kg/day) against MeHg-induced behavioral dysfunction (1.2 mg/kg/day) was age-dependent. Nimodipine and other CCBs have also been shown to attenuate or block selective signs of normal aging in animal models [33–38] but a full characterization of the interaction between MeHg exposure and aging is lacking.

The current study assessed wheel-running and rotarod performance in two age cohorts of male BALB/c mice chronically exposed to either 0 or 10.0 ppm MeHg via daily drinking water and either 0 or 200 ppm nimodipine *via* daily chow. A log-survivor bout analysis approach was used to parse wheel-running into bout of running separated by pauses, which provided parameter estimates of the rate at which bouts occurred (bout-initiation rate), the speed of running within a bout (within-bout rate), and the length of a given bout (bout length) [39]. To account for age-related differences in behavior and attrition due to MeHg toxicity, individual raw data from wheel-running and rotarod performance was compared against the mean of the age-matched control group on a session-by-session basis (standardized Z-score units) see also [26].

2. Methods

2.1. Subjects

Adult and retired breeder male BALB/c mice ($N = 112$) were purchased from Harlan Laboratories (Indianapolis, IN) and housed in an Optimice® rack system in an AAALAC-accredited temperature- and humidity-controlled vivarium that was maintained on a 12-h light-dark cycle (lights on at 6:00am). Two age cohorts, two MeHg water concentrations, and two nimodipine diets produced a 2 (age) × 2 (MeHg) × 2 (nimodipine) full factorial design with 12–16 mice per exposure group by age. Prior to exposure, animals' motor function was tested by assessing wheel-running and rotarod performance (one session each) to ensure preexisting differences did not exist among groups within each age group. Then they were randomly assigned to exposure groups with the constraint that the groups were similar on pre-exposure wheel-running and rotarod performance.

The adult cohort ($n = 51$) arrived at 49 days of age. Mice were housed in pairs in clear polycarbonate cages, separated by a clear Plexiglas© divider that prevented physical contact, but allowed visual, olfactory, and auditory interaction. Adult BALB/c mice are aggressive and group housing frequently results in serious injury or death [40]. Their weight was maintained at approximately 24–25 g and after 4 months transitioned to a final target weight of approximately 26–27 g.

The retired breeder age cohort ($n = 63$) arrived at 273 days of age. Mice were housed in the same manner as the adults. Upon arrival, they weighed 26–30 g, which was reduced and maintained at a final target weight of approximately 26–27 g.

2.2. Methylmercury and nimodipine exposure

Methylmercuric chloride (CH_3HgCl) was procured from Alfa-Aesar (Ward Hill, MA, USA) and dissolved into water to produce the water solutions. Nimodipine was procured from Sigma-Aldrich (St. Louis, MO) and 200 ppm nimodipine was mixed into standard rodent chow manufactured by Purina TestDiets 5LL2 laboratory chow diet. Based on measurements of water and food consumption and weight, MeHg exposure was approximately 0 and 1.2 mg/kg/day of Hg and nimodipine exposure was approximately 0 and 20.0 mg/kg/day. Exposures began when adults and retired breeders were 72 and 296 days old, respectively, and lasted 262 days until the groups were 334 and 558 days old, respectively (see Fig. 1 for a timeline of events).

2.3. Apparatus

Wheel-running sessions were conducted in 16 standard Med Associates Inc. operant conditioning chambers (St. Albans, VT, product #ENV-007). Each chamber measured 30.5 cm L × 24.1 cm W × 29.2 cm H and contained an in-chamber activity wheel (product #ENV-043A). Responses were recorded as quarter-wheel revolutions (approximately 13.95 cm) with centisecond resolution in the measurement of the time between each quarter-wheel displacement. Operant chambers were enclosed in sound-attenuating cabinets with a fan to circulate air for ventilation. Rotarod sessions were conducted using a standard 5-station Med-Associates© Rotarod for mice (product #ENV-575M) during which the speed of the rotating cylinder accelerated from 4 to 40 rpm over 5-min at a constant acceleration of 0.12 revolutions per sec. An infrared beam below the rotating rod detected when an animal fell.