Behavioural Brain Research 311 (2016) 147–159

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Research report

A bout analysis reveals age-related methylmercury neurotoxicity and nimodipine neuroprotection

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HIGHLIGHTS

- Young- and older adult mice were exposed chronically to MeHg and/or nimodipine.
- MeHg-induced mortality was age-independent.
- Nimodipine afforded greater protection against MeHg-induced mortality in younger mice.
- MeHg affected the maximum rate of nose-poking but not the motivation to do so.
- Older mice had a shorter latency to impairment.
- Nimodipine delayed motor deficits by 100+ days in younger compared with older mice.

ARTICLE INFO

Article history:
Received 17 February 2016
Received in revised form 10 May 2016
Accepted 14 May 2016
Available online 16 May 2016

Keywords:
Aging
Methylmercury
Nimodipine
Calcium homeostasis
Bout analysis
Motor function

ABSTRACT

Age-related deficits in motor and cognitive functioning may be driven by perturbations in calcium (Ca^{2+}) homeostasis in nerve terminals, mechanisms that are also thought to mediate the neurotoxicity of methylmercury (MeHg). Calcium-channel blockers (CCBs) protect against MeHg toxicity in adult mice, but little is known about their efficacy in other age groups. Two age groups of BALB/c mice were exposed to 0 or 1.2 mg/kg/day MeHg and 0 or 20 mg/kg/day of the CCB nimodipine for approximately 8.5 months. Adults began exposure on postnatal day (PND) 72 and the retired breeders on PND 296. High-rate operant behavior was maintained under a percentile schedule, which helped to decouple response rate from reinforcer rate. Responding was analyzed using a log-survivor bout analysis approach that partitioned behavior into high-rate bouts separated by pauses. MeHg-induced mortality did not depend on age but nimodipine neuroprotection was age-dependent, with poorer protection occurring in older mice. Within-bout response rate (a marker of sensorimotor function) was more sensitive to MeHg toxicity than bout-initiation rate (a marker of motivation). Within-bout rate declined almost 2 months prior to overt signs of toxicity for the MeHg-only retired breeders but not adults, suggesting greater delay to toxicity in younger animals. Motor-based decrements also appeared in relatively healthy adult MeHg + NIM animals. Aging appeared to alter the processes underlying Ca^{2+} homeostasis thereby diminishing protection by nimodipine, even in mice that have not reached senescence. The study of MeHg exposure presents an experimental model by which to study potential mechanisms of aging.

1. Introduction

Methylmercury (MeHg) is a global pollutant and the primary concerns about its health effects are due to its neurotoxicity [1]. Prenatal exposures produce diffuse central nervous system (CNS) damage and cognitive dysfunction [2–4] whereas adult-onset exposures produce relatively focal damage that appears in the primary motor cortex, sensory regions of the cerebral cortex,

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http://dx.doi.org/10.1016/j.bbr.2016.05.032
0166-4328/

cerebellar granule cells, and dorsal root ganglion and results in motor dysfunction [5–7]. Methylmercury-induced disruptions of intracellular signaling and cell death have been linked, at least in part, to dysregulation of Ca^{2+} homeostasis inside nerve terminals [8,9]. Similarly, neuronal degeneration during aging is thought to be mediated by changes in the level of intracellular Ca^{2+} [10–12]. Chronically elevated levels of intracellular Ca^{2+} in neurons and reduced ability to buffer Ca^{2+} levels during normal aging provoke subtle age-associated declines and mild impairment [12–16]. Chronic MeHg exposure, acting to disrupt Ca^{2+} homeostasis, may exacerbate age-related declines in motor or cognitive functioning and accelerate normal or neurodegenerative aging, as has been noted with MeHg [17].

The excess intracellular Ca^{2+} produced by MeHg and aging suggests that preventing increased Ca^{2+} influx into intracellular cytosol could be neuroprotective. Calcium channel blockers reduce intracellular Ca^{2+} by blocking Ca^{2+} channels located in neuronal cell membranes, cerebral and peripheral vasculature, and cardiac smooth muscle [18–21]. Nimodipine, a 1,4-dihyrdopyridine CCB, is an L-type Ca^{2+} blocker with excellent selectivity for the CNS [22], and is an ideal candidate to protect against Ca^{2+}-mediated CNS insults like MeHg exposure. In vitro [23,24] and in vivo [24–26] studies support this notion. For example, Bailey et al. [25] and Hoffman & Newland [26] found that chronic nimodipine (2–20 mg/kg/day) afforded dose-dependent neuroprotection in adult BALB/c mice chronically exposed to 2.6 mg/kg/day MeHg. Nimodipine attenuated or blocked deficits in an incremental repeated acquisition (IRA) procedure [25], wheel-running and rotarod performance, and mortality [26]. CCBs, including nimodipine, also attenuate or block selective signs of normal aging [27–31] and other CNS insults [32–37]; c.f. [38].

It is difficult to separate motor from motivational components of behavior in models of neurotoxicant-induced motor deficits because the behavior is closely coupled to the motivation to engage in it [39]. Procedures that produce high-rate responding, such as fixed-ratio, variable-ratio, and differential reinforcement of high rate schedules (DRH) inherently link reinforcement rate to response rate. Thus, impairment may produce a positive feedback loop wherein response rate decrements drive reductions in reinforcer rate which could, in turn, further reduce response rate, confounding motor deficits with the consequences of reinforcer loss. In the current study we separated motor and motivational influences first by manipulating the contingency linking responding to the delivery of reinforcers and second by using an analytical approach capable of differentially estimating the contribution of motoric and motivational components of behavior may be advantageous.

We used both percentile (PCNT) and differential reinforcement of high rate (DRH) schedules to maintain high-rate nose-poking. The PCNT schedule is particularly appealing because it titrates the response criterion in real-time according to the subject's recent performance [40,41]. As response rate declines, a PCNT schedule relaxes the response criterion, making it easier to obtain reinforcers. This allows behavior to contact reinforcement even in the face of impairment, disentangling the effect of reinforcer loss on response rate with MeHg- or aging-induced decreases in responding. In contrast, response rate decrements under the DRH schedule generally lead to a direct decrease in reinforcer delivery. To separate further reinforcer rate from motor deficits, criterion responses were reinforced under a random interval 30 s (RI 30 s) schedule of reinforcement, which randomly reinforced criterion response patterns at an average rate of two reinforcers per min.

The analytical approach to separating influences over behavior was based on the observation that high-rate behavior typically occurs as bouts of response bursts separated by intervals during which the animal is disengaged from the target behavior, is to use a dynamic analysis that breaks a response epoch second-by-second into bouts. [42–47]. The key response unit on which this analysis is based is the interresponse time (IRT). A bout comprises a run of short IRTs while the initiation of a new bout typically terminates a long IRT. We used a log-survivor analysis, described in detail by Shull and colleagues [42–44], to partition these IRTs into two distinct distributions. The short IRTs produced by response bursts, or within-bout responses, serve as an index of motor function. In contrast, the long IRTs that represent inter-bout intervals, the inverse of which is bout-initiation rate, serve as an index of the motivation to engage in the target behavior. These interpretations are supported empirically by studies that show that changes in motivating operations like food deprivation selectively affect bout-initiation rate [41,47] whereas manipulations that makes responding more difficult [45] or compounds like MeHg [26] and pentobarbital [46] with known motoric effects preferentially affect within-bout rate. This analysis assumes that responding can be described as three orthogonal components, within-bout rate, bout-initiation rate, and bout length [42–44], which is supported by Hoffman & Newland's [26] reconstruction of overall response rate in control and MeHg-exposed mice by a linear combination of these three terms derived from a change-point analysis.

The present study used a log-survivor bout analysis approach to disentangle motoric from motivational deficits in high-rate nose-poking induced by chronic MeHg exposure and neuroprotection by nimodipine in two age cohorts of male BALB/c mice.

2. Material and 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.

2.1.1. Adults

The adult cohort ($n = 51$) arrived at 49 days of age. Upon arrival, 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. Due to the aggressiveness of adult male BALB/c mice [48], animals remained separated for the duration of the study. Their weight was maintained at approximately 24 g by feeding approximately 2.5 g standard rodent chow per animal per day, adjusted according to their body mass, with free access to water except during experimental sessions. After 4 months, adults transitioned to a final target weight of approximately 26 g by feeding approximately 3.0 g rodent chow per day.

2.1.2. Retired breeders

The retired breeder age cohort ($n = 63$) arrived at 273 days of age and 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 g by feeding approximately 3.0 g standard rodent chow per animal per day with free access to water except during experimental sessions.

2.2. Methylmercury and nimodipine exposure

Methyl mercuric chloride (CH_3HgCl) was procured from Alfa-Aesar (Ward Hill, MA, USA) and dissolved into water to produce