Neurotoxicology and Teratology 59 (2017) 16–26

Contents lists available at ScienceDirect

Neurotoxicology and Teratology

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

Full length article

Developmental manganese neurotoxicity in rats: Cognitive deficits in allocentric and egocentric learning and memory

Robyn M. Amos-Kroohs [a], Laurie L. Davenport [b], Nina Atanasova [c], Zuhair I. Abdulla [d], Matthew R. Skelton [a], Charles V. Vorhees [a], Michael T. Williams [a,*]

[a] Division of Neurology, Cincinnati Children's Research Foundation and University of Cincinnati College of Medicine, Cincinnati, OH 45229, United States
[b] Department of Environmental Health, University of Cincinnati, 3223 Eden Ave., Cincinnati, OH 45220, United States
[c] Department of Philosophy and Religious Studies, University of Toledo, Toledo, OH 43606, United States
[d] Graduate Program in Neuroscience, University of Cincinnati College of Medicine, Cincinnati, OH 43267, United States

ARTICLE INFO

Article history:
Received 10 August 2016
Received in revised form 10 October 2016
Accepted 12 October 2016
Available online 15 October 2016

Keywords:
Manganese
Neurotoxicity
Spatial learning
Egocentric learning
Alpha-synuclein
Long-term potentiation

ABSTRACT

Manganese (Mn) is an essential element but neurotoxic at higher exposure levels. The effects of Mn overexposure (MnOE) on hippocampal and striatal-dependent learning and memory in rats were tested in combination with iron deficiency (FeD) and developmental stress that often co-occur with MnOE. Moderate FeD affects up to 15% of U.S. children and developmental stress is common in lower socio-economic areas where MnOE occurs. Pregnant Sprague-Dawley rats and their litters were housed in cages with or without (barren cage (BAR)) standard bedding from embryonic day (E)7 to postnatal day (P)28. Dams were fed a 90% FeD or iron sufficient (FeS) diet from E15-P28. Within each litter, separate offspring were treated with 100 mg/kg Mn (MnOE) or vehicle (VEH) by gavage on alternate days from P4-28. Offspring were tested as adults in the Morris and Cincinnati water mazes. FeD and developmental stress interactively impaired spatial learning in the Morris water maze. Developmental stress and MnOE impaired learning and memory in both mazes. MnOE resulted in reduced CA1 hippocampal long-term potentiation (LTP) and increased levels of α-synuclein. Preweaning MnOE resulted in cognitive deficits on multiple domains of learning and memory accompanied by impaired LTP and α-synuclein changes, effects worsened by developmental stress.

1. Introduction

Manganese (Mn), an essential nutritional element, is neurotoxic at high levels. First identified in workers following chronic inhalation exposure (Mena et al., 1967), it results in manganism, a disorder that shares some symptoms with parkinsonism. Mn overexposure (MnOE) is also seen in children and is more subtle than manganism (Bouchard et al., 2011; Khan et al., 2011; Khan et al., 2012; Lucchini et al., 2012). In children, the route of exposure can be ingestion from water (Wasserman et al., 2006; Oulhote et al., 2014), soy-based infant formulas (Tran et al., 2002a), and contaminated air and water from smelting factories (Menezes-Filho et al., 2014). Neonates that ingest Mn from infant formula retain more Mn than those breastfed (Dorner et al., 1989). MnOE in children exhibit effects such as cognitive deficits, behavioral disinhibition, and reduced school achievement (Zoni and Lucchini, 2013; Haynes et al., 2015).

In rodent models of developmental MnOE, effects include decreased passive avoidance retention, attenuated locomotor responses to cocaine, impaired rotorod coordination, and reduced striatal dopamine (Tran et al., 2002a; Tran et al., 2002b; Reichel et al., 2006; Cordova et al., 2012). Furthermore, deficits were found in rats exposed to 25 or 50 mg/kg/day Mn by gavage from postnatal day (P)1-21 with MnOE animals taking longer and making more errors than vehicle (VEH) controls in a radial arm maze (RAM) (Kern et al., 2010), an effect not seen in a study using the Morris Water Maze (MWM) or on RAM in rats exposed to 2 or 10 mg/mL in drinking water from embryonic day (E)1 through P30 (Pappas et al., 1997). Two studies show that early MnOE in rats results in reduced fine motor control on a food reaching task (Beaudin et al., 2013; Beaudin et al., 2015).

Developmental MnOE seldom occurs in isolation (Walker et al., 2011). Concomitant factors include impoverished/low socioeconomic status (SES) environments where iron deficiency (FeD), and/or stress (developmental stress) are common. FeD occurs in 15% of U.S. children (Lee and Okam, 2011) and can affect brain development (Youdim, 2008). SES is often used as a surrogate for impoverishment and stress. Children from low SES environments exhibit higher rates of anxiety, conduct disorders, and attention deficit disorders (Hackman et al.,

* Corresponding author at: Division of Neurology (MLC 7044), Cincinnati Children's Research Foundation, 3333 Burnet Ave., Cincinnati, OH 45229-3039, United States.
E-mail address: michael.williams@cchmc.org (M.T. Williams).

http://dx.doi.org/10.1016/j.ntt.2016.10.005
0892-0362/

2010; Walker et al., 2011; Reiss, 2013). The intersection of MnOE, FeD, and low SES/stress may represent a combinatorial risk greater than each factor when acting separately (Wasserman et al., 2006). We hypothesized that cognitive development may be vulnerable to such an interaction since each affects brain regions known to be involved in learning and memory.

In this study, rats were exposed to 100 mg/kg Mn or VEH every other day from P4-28 (Amos-Kroohs et al., 2016). This regimen increases blood and neostriatal Mn and alters monoamine neurotransmitters (Vorhees et al., 2014). FeD was induced using a diet with 90% less Fe than a standard diet. The diet was given from E15-P28 and produced decreased blood hematocrits, body weight, and locomotor activity, all recognized markers of FeD. Moreover, MnOE and FeD in combination increase body weight reductions and alter anxiety in rats compared to either factor alone (Amos-Kroohs et al., 2015). Developmental stress was induced using a barren cage procedure (Avishai-Eliner et al., 2001; Vorhees et al., 2014). Rats were placed in barren (BAR) cages from E7-P28, an interval spanning most of neurogenesis (Clancy et al., 2007). After cessation of exposure, offspring were tested for cognitive ability using tests for allocentric and for egocentric learning and memory. Allocentric learning and memory was evaluated using the MWM (Morris et al., 2003; Vorhees and Williams, 2006), a test that relies on the use of extramaze cues to determine the shortest path from start to goal (a hidden platform). Egocentric learning and memory was evaluated using the Cincinnati water maze (CWM) (Vorhees, 1987; Vorhees et al., 1991; Braun et al., 2012; Braun et al., 2015). In this test, animals must use internal, self-movement cues to find the goal in a complex labyrinthine maze in complete darkness. Separate, identically treated animals were used to assess α-synuclein and long-term potentiation (LTP), the latter an established correlate of spatial learning and memory (Lisman et al., 2002; Morris et al., 2003). α-Synuclein was assessed based on its association with dopamine and Lewy body formation associated with parkinsonism.

2. Materials and methods

2.1. Animals

Nulliparous female Sprague-Dawley CD (IGS) rats (Charles River Laboratories, Raleigh, NC; strain #001), approximately 60 days old on arrival were habituated for not less than one week to the vivarium (AAALAC International accredited) before breeding by being placed with male sires of the same strain and supplier. Animals were maintained on a 14–10 h light-dark cycle (lights on 600 h) with controlled temperature (19 ± 1 °C) and humidity (50% ± 10%). Animals were housed in a barrier facility using a Modular Caging System (Alternative Design, Siloam Spring, AR). HEPA filtered air was supplied to each cage (Alternative Design, Siloam Spring, AR) with 30 air changes/h. Reverse osmosis filtered water (SE Lab Group, Napa, CA) and NIH-07 diet were provided ad libitum. A semicircular stainless steel enclosure was placed in standard cages for enrichment (Vorhees et al., 2008). Females were separated the day a sperm plug was detected and this day was designated E0. Birth was counted as P0; on P1, litters were culled to 10, five per sex, using a random number table. Pups were removed from dams on P28 into same sex cages (4/cage) and re-housed (2/cage) on P42. Maternal body weight was measured on E7, 15, 21, and P1 and 28. Pups were weighed on P1, during dosing and on P42 and P60.

2.2. Rearing

Gravid females were housed in standard cages (STD) until E7 at which time half were moved to cages without bedding or enclosures and with a wire grid floor inserted (BAR $n = 29$); the other half were continued in STD cages ($n = 30$) but moved to new STD cages on E7 to control for rehousing experience. On E21, wire floors were temporarily removed to prevent pups from slipping through the spaces between wires of the grid floor, and a 15 × 25 cm absorbent pad was placed in each cage instead (Anderson Lab Bedding, Maumee, OH). On *P*6, BAR cages had pads removed and grid floors reinstalled. Cages were changed daily for both types of litters. BAR cages were maintained until P28 then switched back to STD cages.

2.2.1. Diet

The FeD diet (Amos-Kroohs et al., 2015) was adapted from (Fitsanakis et al., 2009; Fitsanakis et al., 2011). Females were given standard NIH-07 diet until E15 then switched to purified NIH-07 diet (Land O' Lakes Purina Feed, Evansville, IN) with half of the dams in the BAR and STD groups given purified iron sufficient (FeS) diet and the other half purified FeD formulated diet. The FeD diet contained 35 ppm Fe and the FeS diet contained 350 ppm Fe (the standard NIH-07 diet also contains sufficient Fe). Offspring were returned to standard NIH-07 diet on P28.

2.3. Manganese

For MnOE (Vorhees et al., 2014), a split-litter design was used in which two male and two female pups per litter were gavaged with VEH (0.01 M anhydrous sodium chloride) and three male and three female pups per litter were gavaged with 100 mg/kg Mn chloride (MnOE). The extra pair of MnOE pups was included only for backup purposes. Gavage solutions were given in a volume of 3 mL/kg of VEH every other day from P4-28. Gavage was used to avoid maternal exposure and its effects on maternal-pup behavior (Graham et al., 2011). This exposure regimen produces increased serum and brain Mn (Amos-Kroohs et al., 2015) but does not increase corticosterone above that of untreated littermates (Graham et al., 2011).

2.4. Behavior

One male-female pair from each exposure group within each litter received one set of all tests shown on the left and one male-female pair from each exposure group received a second set of tests shown on the right in Fig. 1. Only the offspring learning and memory data are included here; data on the other outcomes, including body weights and litter composition, were reported separately (Amos-Kroohs et al., 2016). This design means that prior behavioral tests may have some influence on later tests. For the learning and memory tests, rats in both test sequences received straight water channel testing prior to maze testing. There were a total of 234 rats in the CWM testing arm and 216 rats in the MWM testing arm. This design resulted in approximately 12 offspring from different litters per sex per rearing condition per diet per Mn exposure group.

2.4.1. Straight Channel

This test acclimates rats to swimming, teaches escape to a hidden platform, and tests motivation and swimming coordination by measuring swim speed as latency to traverse a long straight water channel. On P60, animals were placed at one end of a 15 × 244 × 50 cm high channel filled with water to a depth of 25 cm and given four trials to reach the submerged platform at the opposite end. Latency to reach the platform was recorded.

2.4.2. Cincinnati water maze (CWM)

The apparatus is a 9-unit multiple-T labyrinthine water maze (Vorhees, 1987; Vorhees et al., 1991; Vorhees and Williams, 2016). Trials are run under infrared light with a submerged platform at the goal location. By testing in the absence of visible light, distal cues are eliminated which prevents rats from using spatial cues to find the escape. We have shown that learning in this maze under these conditions is severely disrupted by dopaminergic reductions in the neostriatum (Braun et al., 2012; Braun et al., 2015). The maze channels are 15 cm wide and the walls 51 cm high filled with water to a depth of 20 cm. The day after