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Olfactory toxicity in rats following manganese chloride nasal instillation: A pilot study

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ABSTRACT

Following inhalation, manganese travels along the olfactory nerve from the olfactory epithelium (OE) to the olfactory bulb (OB). Occupational exposure to inhaled manganese is associated with changes in olfactory function. This pilot study evaluated two related hypotheses: (a) intranasal manganese administration increases OE and OB manganese concentrations; and (b) intranasal manganese exposure impairs performance of previously trained rats on a go-no-go olfactory discrimination (OD) task. Male Fischer 344 rats were trained to either lever press ("go") in response to a positive conditioned stimulus (CS+: vanillin) or to do nothing ("no go") when a negative conditioned stimulus (CS−: amyl acetate) was present. Following odor training, rats were randomly assigned to either a manganese (200 mM $MnCl_2$) or 0.9% saline treatment group (n = 4–5 rats/group). Administration of either saline or manganese was performed on isoflurane-anesthetized rats as 40 μL bilateral intranasal instillations. Rats were retested 48 h later using the vanillin/amyl acetate OD task, then euthanized, followed by collection of the OE and OB. Manganese concentrations in tissue samples were analyzed by ICP-MS. An additional cohort of rats (n = 3–4/group) was instilled similarly with saline or manganese and nasal and OB pathology assessed 48 h later. Manganese-exposed rats had increased manganese levels in both the OE and OB and decreased performance in the OD task when compared with control animals. Histopathological evaluation of the caudal nasal cavity showed moderate, acute to subacute suppurative inflammation of the olfactory epithelium and submucosa of the ethmoid turbinates and mild suppurative exudate in the nasal sinuses in animals given manganese. No histologic changes were evident in the OB. The nasal instillation and OD procedures developed in this study are useful methods to assess manganese – induced olfactory deficits.

1. Introduction

An association between manganese inhalation and neurotoxicity was first recognized in the mid-19th century in workers at an ore-grinding plant where "black oxide of manganese" was processed (Couper, 1837). Since then a number of other sources of high dose manganese exposure have been identified including inhalation in occupational settings, such as mining and welding, consumption of manganese-contaminated drinking water, and in people receiving intravenous total parenteral nutrition (TPN) solutions fortified with additional manganese (Crinella, 2012; Hardy et al., 2008; Michalke et al., 2007). High-dose manganese exposure in people produces a form of parkinsonism that is characterized by manganese accumulation in the basal ganglia, impaired motor control, and loss of dopaminergic neurons (Bowler et al., 2006; Ellingsen et al., 2008; Guilarte, 2013; Roels et al., 2012). A growing epidemiologic literature suggests that chronic exposure to manganese may predispose an individual to acquire an earlier onset of neurodegeneration (Andruska and Racette, 2015; Gorell et al., 1999; Racette et al., 2001; Willis et al., 2010) although the epidemiologic evidence in support of a causal association between manganese exposure and Parkinson's disease risk remains inconclusive (Mortimer et al., 2012; Wirdefeldt et al., 2011).

There are several mechanisms by which manganese can reach the central nervous system (CNS). Direct "nose-to-brain" transport of manganese via olfactory endings (i.e., olfactory transport) located in the nasal cavity has received increased attention among toxicologists (Lucchini et al., 2012a,b). Early evidence in favor of

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direct olfactory transport of manganese was derived from studies that followed the movement of radiolabeled manganese in freshwater fish after ^{54}Mn was instilled in the olfactory cavity (Tjälve et al., 1995; Tjälve and Henriksson, 1999). Since that time, other investigators have confirmed olfactory transport occurs in rodents following manganese inhalation (Brenneman et al., 2000; Elder et al., 2006). The results of these studies are consistent with a number of studies that have evaluated the pharmacokinetics of manganese in animals following manganese inhalation and showed accumulation of manganese in the olfactory bulb (OB) (Dorman et al., 2004; Fechter et al., 2002; Normandin et al., 2002, 2004; Salehi et al., 2003; St-Pierre et al., 2001; Tapin et al., 2006; Vitarella et al., 2000). For example, rhesus monkeys and rats exposed to manganese sulfate at either 0.06 or 0.1 mg Mn/m^3, respectively, for 65 exposure days developed an approximate doubling in OB manganese concentrations (Dorman et al., 2004, 2006). Neuroimaging studies in animals that followed the movement of manganese further confirm the anterograde movement of this metal (Cross et al., 2004; Dorman et al., 2006; Murayama et al., 2006; Saleem et al., 2002). Rats given manganese by gavage also develop increased OB manganese concentrations, presumably by mechanisms distinct from olfactory transport (Foster et al., 2015).

Concerns have been raised regarding risks associated with olfactory transport of manganese in people (Lucchini et al., 2012a, b). Olfactory transport of manganese appears to be well conserved among animals including fish and mammals suggesting this pathway is likely operative in people. Sen et al. (2011) used brain MRI to evaluate region-specific manganese accumulation in seven welders without obvious neurologic deficits. When compared with age- and gender-matched controls, the manganese-exposed welders had significantly higher T1 relaxation rates in the OB as well as evidence of manganese accumulation in the frontal white matter, globus pallidus, and putamen. Although these results are consistent with animal studies evaluating olfactory transport of manganese, they do not provide direct evidence that olfactory transport of manganese occurs in humans.

There are additional concerns whether olfactory transport of manganese may contribute to neurologic dysfunction – especially effects on the sense of smell. Welders exposed to manganese may develop a decreased sense of smell (hyposmia) and other olfactory disorders (Antunes et al., 2007). To date few studies have assessed whether manganese delivery via the olfactory route impairs the function of olfactory neurons. For example, Moberly et al. (2012) showed that acute intranasal manganese administration in mice caused a 90% reduction in odorant-evoked neurotransmitter release. Another study showed that, during the early period following intranasal manganese administration, odor-related behaviors were dose-dependently reduced in rats (Lehallier et al., 2012). This study explores the effects of manganese on the mammalian olfactory system using a combination of pharmacokinetic, behavioral, and histologic methods to assess changes in the olfactory system of rats following intranasal instillation of an acute high dose of manganese.

2. Materials and methods

2.1. Experimental design

One phase of the study assessed the effect of manganese exposure on a previously trained odor discrimination task. Fig. 1 illustrates the main phases used to complete the olfactory discrimination test that was used in the study. Once animals had acquired the olfactory discrimination task, pairs of trained rats were randomly assigned to either saline or manganese treatment groups, intranasal exposures were completed under anesthesia, and performance on the olfactory discrimination task was reassessed 48 h after chemical exposure. We also evaluated olfactory bulb and olfactory epithelium manganese concentrations in these animals. An additional cohort of rats was instilled similarly with saline or manganese and nasal and olfactory bulb pathology assessed 48 h later.

2.2. Chemicals

Manganese (Mn^{2+}) chloride ($MnCl_2{\cdot}4H_2O$; ≥99%) was obtained from Sigma Aldrich Chemical Corporation (St. Louis, MO). Manganese chloride is a crystalline powder that contains 27.3% manganese by weight and is relatively soluble in water. Unless otherwise indicated, all other chemicals were obtained from Sigma Aldrich Chemical Corporation (St. Louis, MO) or Fisher Scientific (Pittsburgh, PA).

2.3. Animals and animal husbandry

This study was conducted under federal guidelines for the care and use of laboratory animals (National Research Council, 2011) and approved by the North Carolina State University (NCSU) Institutional Animal Care and Use Committee. Male 8 week old F344 rats (Charles River, Kingston, NY) were purchased for the study. Rats were quarantined (1 week) and housed in the AAALAC-accredited animal facility at the College of Veterinary Medicine's Laboratory Animal Resources (LAR) facility using standard caging systems and appropriate materials for enrichment. Animal rooms were maintained at daily temperatures of 22 ± 4 °C, relative humidity of 30–70%, and an air flow rate sufficient to provide 10–15 air changes per hour. Fluorescent lighting was controlled by automatic controls (lights on approximately 0700–1900). On arrival, rats were pair housed in polycarbonate cages and provided food and water ad libitum. A pelleted, LabDiet 5001 diet (Richmond, IN) was fed to all animals. During quarantine the rats were uniquely identified using tail marks.

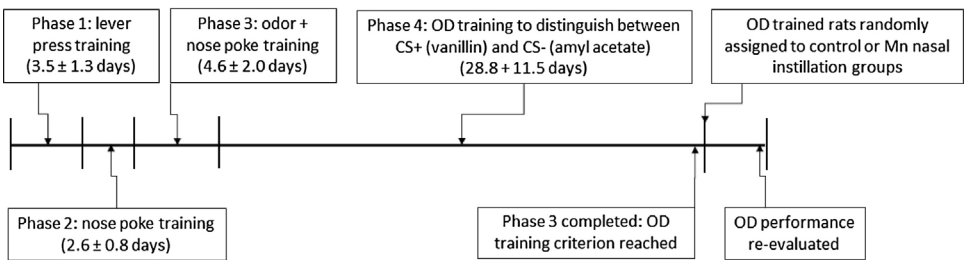

Fig. 1. Overview of phases used to train rats on the vanillin:amyl acetate olfactory discrimination (OD) task. Once animals reached criteria on the OD task they were randomly assigned to either control or manganese exposure groups. Animal performance on the OD task was reassessed 48 h after saline (control) or manganese exposure by nasal instillation. Mean (±SEM) number of daily sessions to complete each training phase is also provided (see text for additional details).

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