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# Effect of manganese and manganese plus noise on auditory function and cochlear structures

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ABSTRACT

The degenerative actions of Mn caused by persistent exposure to high atmospheric levels not only provokes irreversible damage to the CNS with symptoms comparable to that of Parkinson's disease but also may have deleterious consequences to other organs including the auditory system. The putative deleterious consequences of prolonged Mn overexposure on hearing, however, is confounded by the fact that chronically-exposed individuals often work in high noise environments where noise by itself is known to cause hearing loss. Thus, the question as to whether Mn alone is actually ototoxic and whether exposure to Mn when combined with noise increases the risk of hearing loss and cochlear pathology has never been examined. To examine whether noise effects Mn ototoxicity, we exposed rats to a moderate dose of Mn (10 mg $MnCl_2$/liter water) alone, a high level of noise (octave band noise, 8–16 kHz, presented at 90 dB SPL for 8 h/d) alone or the combination of Mn plus noise and measured the changes in auditory function and the cochlear histopathologies. Results of these studies, based on various measures of hearing including histological examination of cochlear tissue suggest that noise alone produced significant hearing deficits whereas semi-chronic exposure to moderate levels of Mn in drinking water for 90 days either in the presence or absence of noise had, at best, only a minor effect on hearing.


## 1. Introduction

Although manganese (Mn) is an essential transition metal required for normal health, high atmospheric levels in various occupational settings can lead to a syndrome called manganism (Racette, 2014; Roth, 2006). Initial neurological symptoms consist of reduced response speed, irritability, intellectual deficits, mood changes, and compulsive behaviors whereas prolonged exposures lead to more severe deficits within the extrapyramidal system that includes dystonic movements associated with Parkinson's disease, a masklike face, limb rigidity, mild tremors, gait disturbance, slurred speech, excessive salivation, sweating and a marked disturbance of balance. Other patients with chronic liver disease display increased Mn level in serum and brain, as well as behavioral deficits and neurodegenerative features resembling those seen in Mn-exposed workers because the liver is the major organ responsible for its elimination. The behavioral components, also seen upon Mn intoxication, suggest that Mn may affect a variety of neurotransmitter systems in the central nervous system (CNS) outside of its putative target, the globus pallidus (Bowler et al., 2006; Roels et al., 2012). Mn overexposure can lead to disturbances in dopaminergic (Higashi et al., 2004; Peneder et al., 2011; Roth et al., 2013), glutamatergic (Erikson and Aschner, 2002; Erikson and Aschner, 2003; Guilarte and Chen, 2007; Roth et al., 2012), GABAergic (Anderson et al., 2008; Burton et al., 2009; Erikson and Aschner, 2003) and cholinergic systems (Finkelstein et al., 2007) in the CNS. Although the neurobehavioral complications associated with chronic Mn exposure were thought to be reversible, more recent studies indicate that some symptoms are permanent (e.g., neuromuscular function, cognitive flexibility, and adverse mood states (Bowler et al., 2011; Roels et al., 1999).

The degenerative effects associated with persistent Mn exposure may have deleterious consequences to other organs including the auditory system (Antonini et al., 2003; Antonini et al., 2009; Da Silva et al., 2007; Ding et al., 2011; Josephs et al., 2005; Khalkova and Kostadinova, 1986; Korczynski, 2000; Zeidler-Erdely et al., 2011). However, the putative deleterious consequences of prolonged Mn overexposure is confounded by

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the fact that chronically-exposed individuals often work in high noise environments where noise by itself is known to cause hearing loss. For example, several studies have reported that both welders and miners, who are exposed to high levels of Mn and noise, acquire irreversible hearing impairment (Da Silva et al., 2007; Gratton et al., 1990; Josephs et al., 2005, Khalkova and Kostadinova, 1986; Korczynski, 2000; Park et al., 2006). Since noise by itself can cause hearing loss the key question is whether the hearing loss reported in these complex environments is caused by Mn alone, noise alone or the combination of these two agent. This issue is critical important because previous studies have shown that noise exposure exacerbates hearing loss and cochlear pathology induced by a variety ototoxic agents such as aminoglycoside antibiotics, cisplatin, carbon monoxide, carbon disulfide, toluene and heavy metals including lead, mercury, cadmium, and arsenic (Brown et al., 1978; Counter and Buchanan, 2002; Gratton et al., 1990; Hwang et al., 2009; Prasher, 2009). Our *in vitro* studies with postnatal cochlear organotypic cultures (Ding et al., 2011) clearly indicate that μM levels of Mn can damage hair cells and spiral ganglion neurons (SGN). *In vitro*, Mn induced an atypical pattern of damage; SGN were more vulnerable to Mn than hair cells and among the two hair cell populations, inner hair cell (IHC) damage was greater than outer hair cells (OHC). A recent paper (Mirzaee et al., 2007) measured the effect of welding fumes and noise on the function of OHCs in rabbits by examining DPOAE. Rabbits exposed to noise at 110 dB SPL for 8 h/day for 12 days showed significant reductions in DPOAE amplitudes which were further reduced when combined with inhalation of welding fumes that contained high level of Mn plus other metals and gases. These findings suggest that welding fumes, containing a mixture of metals and gases, have the potential to exacerbate noise-induced hearing loss. Unfortunately, no histological measurements were performed to characterize the damage to hair cells and SGN.

Mn can be taken up from the bloodstream and into tissues by metal transporters such as DMT1, ZIP8 and ZIP14; these metal transporters have been identified in the inner ear by quantitative RTPCR and immunohistochemistry (Ding et al., 2014; Ma et al., 2008). Mn levels in the whole otic capsules including the surrounding bone increased roughly 10 fold after intraperitoneal administration of $MnCl_2$ every other day for three days. Mn levels were still four fold greater than controls 14 d after treatment was discontinued. Mn can also be ingested from food and dietary supplements and chronic treatment could increase the levels of Mn in the brain and cochlea. By carefully dissecting out separate regions of the cochlea, we found that Mn concentrations in the basilar membrane, stria vascularis and modiolus of the cochlea increased by 73%, 62% and 27% respectively when rats were fed $MnCl_2$ in drinking water (10 mg/ml) for 30 d (Mullin et al., 2015). Similar increases in Mn were seen in these three subdivisions of the cochlea when the duration of $MnCl_2$ treatment was extended to 60 d suggesting that Mn levels had reached a plateaus after 30 days (Mullin et al., 2015). Mn levels were also measured in the inferior colliculus, located in the auditory midbrain, the striatum and globus pallidus. With the exception of the GP, there was a significant increase in Mn levels in all brain areas in animals treated with Mn for both the 30 and 60 days.

Taken together, these *in vitro* and *in vivo* studies suggest that exogenous Mn accumulates in the cochlea; that Mn alone may be ototoxic and that combined exposure to Mn plus noise increases the risk of hearing loss and cochlear pathology over that caused by noise alone. To test these hypotheses, we exposed rats to drinking water containing a high dose of Mn alone, a high level of noise alone or the combination of Mn plus noise and measured the changes in auditory function and the cochlear histopathologies.

## 2. Materials and methods

### 2.1. Animals

The twenty-four male Sprague Dawley rats (2 months of age, Charles River Laboratories Inc.) used in this study were housed in the Laboratory Animal Facility (LAF) at the University at Buffalo and given free access to food and drinking water (see details of water during experimental treatment described below). The colony room was maintained at 22 °C with a 12-h light-dark cycle. All procedures used in this project were approved by the Institutional Animal Care and Use Committee (HER05080Y) at the University at Buffalo and carried out in accordance with NIH guidelines.

### 2.2. Mn treatment and noise exposure

The rats were randomly divided into 4 groups (n = 6 per group). Each animal in the four groups was housed in a separate cage during the experiment in order to monitor the water and Mn intake. The rats in the control group (Ctrl) were provided with flavored water (1 g unsweetened Kool-Aid plus 1.5 g saccharin per liter water) ad libitum for 90 d in their home cage in a conventional room in the animal facility. Rats in the Mn group (Mn) were supplied ad libitum for 90 d with the same flavored drinking water as above that also contained 10 mg $MnCl_2$/liter water similar to the procedure of Aliva et al. (Avila et al., 2008). The rats in Mn group were housed in the same room as the Ctrl group. The daily water intake and body weight of each animal were measured every second day. From these measures, we calculated the amount of $MnCl_2$ consumed by each animal over the course of the 90-d treatment. The rats in the noise group (Noise) were exposed to an octave band noise (8–16 kHz) presented at 90 dB SPL for 8 h/d (9:00am – 5:00pm) for 90 d; these Noise group rats received the same flavored water as the Ctrl group. Each rat was noise exposed in its home cage; the noise exposure took place in a separate room in the animal facility. A calibrated loudspeaker (Fostex, FT28 1–50 kHz) was mounted above each cage. The rats in the Mn + Noise group were treated for 90 days with Mn (as above) plus Noise (as above). Water intake and body weight were measured every other day and used to compute the daily $MnCl_2$ intake (as above).

### 2.3. Noise exposure

The noise (8–16 kHz) was generated using a TDT RP2 real time signal processor (TDT, Gainesville, FL), amplified and delivered to a loudspeaker (Vifa D25AG35, Madisound Speaker Components) mounted 8.9 cm above top of each cage. The noise levels in each cage (L = 48.3, W = 25.4, H = 20.3 cm) were measured at the center of the cage directly below the speaker approximately 7.6 cm above the cage floor (*i.e.*, level of the animal's ears); sound levels near the perimeter of the cage were 1–2 dB lower. The sound level was measured with a sound level meter (Larson Davis System 824) equipped with half-inch, free-field condenser microphone (model 2540, Larson Davis).

### 2.4. Distortion product otoacoustic emissions (DPOAE)

Approximately six weeks after termination of the 90-d treatments, DPOAE ($2F_1$–$F_2$) were measured by using the Smart Distortion Product Otoacoustic Emission System (Intelligent Hearing System, version 4.53). The animals were initially anesthetized by inhalation of 4% isoflurane in oxygen at a flow rate of 0.6 l/min and subsequently maintained at 1.5% isoflurane. The earpiece containing a microphone (Etymotic10B+) and two sound delivery tubes was inserted into the ear canal. Two IHS-3738