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R.G. Lucchini
University of Brescia, lucchini@med.unibs.it

D.C. Dorman
North Carolina State University
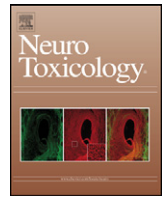
A. Elder
University of Rochester

B. Veronesi
U.S. Environmental Protection Agency Research Triangle Park

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NeuroToxicology

Neurological impacts from inhalation of pollutants and the nose–brain connection

R.G. Lucchini [a,*], D.C. Dorman [b], A. Elder [c], B. Veronesi [d]

[a] Department of Experimental and Applied Medicine, Section of Occupational Health, University of Brescia, Brescia, Italy
[b] College of Veterinary Medicine; North Carolina State University, Raleigh, USA
[c] Department of Environmental Medicine, University of Rochester, Rochester, USA
[d] National Health and Environmental Effects Laboratory, Integrated Systems Toxicology Division, U.S. Environmental Protection Agency Research Triangle Park, USA

ABSTRACT

The effects of inhaled particles have focused heavily on the respiratory and cardiovascular systems. Most studies have focused on inhaled metals, whereas less information is available for other particle types regarding the effects on the brain and other extra-pulmonary organs. We review here the key available literature on nanoparticle uptake and transport through the olfactory pathway, the experimental data from animal and in vitro studies, and human epidemiological observations. Nanoparticles (<0.1 μm in one dimension) may easily reach the brain from the respiratory tract via sensory neurons and transport from the distal alveoli into the blood or lymph as free particles or inside phagocytic cells. These mechanisms and subsequent biologic responses may be influenced by the chemical composition of inhaled particles. Animal studies with ambient particulate matter and certain other particles show alterations in neuro-inflammatory markers of oxidative stress and central neurodegeneration. Human observations indicate motor, cognitive, and behavioral changes especially after particulate metal exposure in children. Exposure to co-pollutants and/or underlying disease states could also impact both the biokinetics and effects of airborne particles in the brain. Data are needed from the areas of inhalation, neurology, and metal toxicology in experimental and human studies after inhalation exposure. An increased understanding of the neurotoxicity associated with air pollution exposure is critical to protect susceptible individuals in the workplace and the general population.

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1. Olfactory transport of inhaled particles and metals

The olfactory system originates with specialized olfactory neurons found within the olfactory epithelium that lines a portion of the nasal cavity. Projections from the olfactory neurons form the olfactory nerve (cranial nerve I), which ultimately terminates in the olfactory bulb after the nerve tracts pass through the skull. Transport of xenobiotics – including particles – along the olfactory nerve provides a route (nose-to-brain) for delivery to the central nervous system (CNS) that bypasses the protective blood brain barrier.

Evaluation of nose-to-brain transport of xenobiotics is fraught with several technical challenges (Dhuria et al., 2010). To date, most experimental studies examining nose-to-brain transport have focused on metals and often used radiolabeled isotopes. Postmortem tissue collection can involve microdissection of the nasal cavity and brain or cryosectioning techniques coupled with autoradiography.

Liquid scintillation, gamma spectrometry, mass spectrometry, proton induced X-ray emission (PIXE), atomic emission or absorption spectroscopy, and other more specialized analytical methods are used to quantify metal concentrations in tissues. The movement of paramagnetic metals can also be followed using brain magnetic resonance imaging (MRI). Some of these techniques are only semi-quantitative. Many experiments rely on intranasal delivery, typically by direct instillation of metal-containing solutions into one nostril. An alternative approach used in inhalation studies involves occluding one nostril, thus restricting olfactory transport to the side of the brain ipsilateral to the patent nostril. The reader is directed to the studies cited in this manuscript for more details concerning these and other methodological approaches.

The association between manganese (Mn) inhalation and neurotoxicity (Guilarte, 2010) has prompted investigators to examine the role of olfactory transport in the delivery of Mn to the brain. Initial studies examining direct nose-to-brain transport of Mn relied on intranasal instillation of [54]Mn (as the soluble chloride salt) and documented the presence of [54]Mn in the olfactory bulb of rats, mice, and freshwater pike on the side ipsilateral to the instilled nostril (Tjälve and Henriksson, 1999). These studies documented that ionic Mn crossed synapses within the olfactory pathway and traveled along secondary and tertiary

* Corresponding author at: Department of Experimental and Applied Medicine Section of Occupational Health, University of Brescia, P.le Spedali Civili 1, 25123 Brescia, Italy. Tel.: +39 030 3996604; fax: +39 030 3996080.
E-mail addresses: lucchini@med.unibs.it, rlucchin@gmail.com (R.G. Lucchini).

neurons to more distal sites within the brain. Brenneman et al. (2000) were among the first to examine this transport route following Mn inhalation. Brenneman used an occluded nostril model and showed that delivery along the olfactory route accounted for nearly all of the ^{54}Mn found in the olfactory bulb and olfactory tract of the rat brain following acute inhalation exposure. Lewis et al. (2005) reported that the rat trigeminal nerve may also deliver Mn from the nasal cavity to the brain.

Olfactory transport rapidly (within 8–48 h) delivers Mn to the olfactory bulb and olfactory tract. However, it appears to be relatively inefficient in delivering inhaled Mn to more distant brain structures. Using brain MRI, Cross et al. (2004) showed that nasally instilled Mn does not undergo transport to the rat striatum or other more distal brain structures. Likewise, Dorman et al. (2006) used brain MRI to demonstrate presumed olfactory transport of inhaled Mn sulfate – which is readily soluble in vivo – in rhesus monkeys. Dorman did not demonstrate evidence for direct translocation of Mn from the olfactory bulb to the globus pallidus, a known target for Mn neurotoxicity.

Much of the experimental evidence in support of the olfactory transport of Mn has come from rodent studies. Unlike people, rodents are obligate nasal breathers and have distinct airway turbinate and epithelial anatomy. These differences may affect Mn delivery to the olfactory epithelium and subsequent brain delivery. Although direct evidence for olfactory transport of Mn in people is currently lacking, this pathway is functional in human beings. Shiga et al. (2010) assessed the transport of nasally administered thallium (^{201}Tl) to the human brain in healthy volunteers using a combination of single photon emission computed tomography (SPECT), X-ray computed tomography (CT), and MRI. These studies demonstrated appreciable movement of Tl to the human olfactory bulb with transport kinetic properties that were most consistent with delivery via the olfactory nerve. These observations are consistent with results from rodent studies demonstrating olfactory transport of Tl (Kanayama et al., 2005), suggesting that the mechanisms involved in olfactory transport of metals are conserved across different species.

Transport mechanisms and the toxicological significance of olfactory transport of Mn remain poorly understood. Henriksson and Tjälve (2000) found that intranasal instillation of Mn resulted in alterations in olfactory bulb expression of glial fibrillary acidic protein (GFAP) and S-100b in rats, both of which are markers of astrocyte activation. In contrast, Dorman et al. (2004) did not observe changes in rat olfactory bulb GFAP concentrations following subchronic inhalation to Mn sulfate, even though olfactory bulb Mn concentrations were increased approximately 3.5-fold versus air-exposed controls. More recently, Villalobos et al. (2009) showed that the mouse olfactory bulb develops neuron degeneration and myelin sheath disorganization following high dose intraperitoneal Mn injection. Recent studies have suggested a role for divalent metal transporter (DMT-1) in Mn olfactory transport with enhanced transport occurring in anemic animals (Thompson et al., 2007).

Although direct nose-to-brain transport has garnered increased attention from neurotoxicologists, it is also important to note that certain metals do not undergo appreciable olfactory transport. For example, inhaled tungsten (Radcliffe et al., 2009) and iron (as the sulfate salt) are poorly transported from the nasal cavity (Rao et al., 2003). Evaluation of this type of toxicokinetic data can be assisted by the use of physiologically based pharmacokinetic models (PBPK) that have been developed to describe olfactory transport of Mn, tungsten, and iron (Leavens et al., 2007).

2. Nanoparticle dosimetry

Air pollution is a complex mixture of gas- and particulate-phase components that has input from natural and anthropogenic sources. The composition of the particulate phase depends on the source(s), aerosol age, and size fraction and can include carbonaceous soot, metals, crustal elements, organics, and biological agents (e.g. pollens). Particles in ambient air exist in four size classes, with the smallest being the ultrafine or nanosized particles (NPs). Nanoparticles are defined as being <0.1 μm in at least one dimension and include natural or anthropogenic/ engineered materials. These particles in an urban atmosphere – when present as singlet, non-agglomerated particles – contribute very little by mass to an exposure, but are by far the most numerous in air (Finlayson-Pitts and Pitts, 2000; Pekkanen et al., 1997) and also have high surface area to volume ratios. NPs are predicted to deposit with high efficiency throughout the entire respiratory tract (ICRP, 1994), including the nasopharyngeal–laryngeal and alveolar regions. Unique anatomical features of these regions include, for the nasal region, the presence of olfactory nerve receptor cells and, for the alveolus, the proximity of the airspace to the vascular bed. The role of the olfactory pathway in the transport of NPs and other xenobiotics to the brain is an emerging issue.

Using laboratory-generated model aerosols, studies of the biodistribution of poorly soluble NPs (carbonaceous, metal) have been performed. These studies have consistently found minute, but statistically significant, accumulation of NPs in extrapulmon-ary tissues, including liver, heart, kidney, spleen, and brain (Kreyling et al., 2009; Oberdörster et al., 2002; Semmler-Behnke et al., 2008). The work of Kreyling and colleagues has, in addition, demonstrated that this response is size-dependent (greater accumulation for smaller particles), but particle type-independent (Kreyling et al., 2009; Semmler-Behnke et al., 2008). Because of the exposure route (intratracheal inhalation), these studies did not directly examine the route by which NPs or their constituents entered the brain.

Work done with elemental carbon NPs (very poorly-soluble) that were delivered to rats by whole-body inhalation exposure demonstrated that the olfactory bulb was a site of NP accumulation (Oberdörster et al., 2002). Uptake and transport by the olfactory nerve or perineural transport could account for the observed uptake. Elder et al. (2006) also employed freshly generated Mn oxide NP-containing aerosols (to simulate welding fume) to expose rats via whole-body inhalation, which led to increases in olfactory bulb, striatum, cortex, and cerebellum Mn content. Although the Mn oxide particles were very poorly soluble, the contribution to the increases in brain from absorption in the deep lung and gastrointestinal tract could not be ruled out. However, when short-term exposures were conducted with the right naris occluded, Mn accumulated only in the left naris. These data support the idea that at least the early Mn accumulation occurs via olfactory transport. This finding is consistent with other studies using more soluble forms of Mn (Brenneman et al., 2000).

2.1. Evidence for air pollution-induced neurotoxicity

Early evidence linking air pollution and neurotoxicity was provided by Calderón-Garcidueñas et al. (2003), who reported neuropathological lesions in feral dogs living in Mexico City.

Inflammation of the olfactory bulb and deficits in olfaction have also been observed in Mexican children residing in highly polluted areas (Calderón-Garcidueñas et al., 2010). Alpha-synuclein neuro-nal aggregation, an early neuropathological hallmark of sporadic Parkinson's disease, and accumulation of 3-nitrotyrosine and 8-hydroxydeoxyguanosine (8-OHdG), evidence of oxidative stress, were also detected in these children's brainstem nuclei (Calderón-Garcidueñas et al., 2011).

As mentioned earlier, the association between inhalation of certain metals and neurotoxicity has also been established. One

case in point is definitely Mn neurotoxicity. For example, motor, cognitive and behavioral functions were assessed in healthy children (11–14 years old) and elderly (65–75 years old) residents in Valcamonica, Italy. In this area, an increased prevalence of Parkinsonism had been observed in relationship with the Mn levels in the deposited dust from ferroalloy airborne emissions (Lucchini et al., 2007). Individual exposure to airborne particles was assessed with 24 h personal sampling and chemical analysis of metal concentration in the filters. Soil metal concentrations were also assessed as a proxy of cumulative exposure from airborne emissions. Several associations were observed between Mn exposure and abnormalities of motor and olfactory functions in both age groups. Regression models showed impairment of motor coordination (Luria-Nebraska test, $p = 0.0005$), hand dexterity (Aiming Pursuit test, $p = 0.0115$) and odor identification (Sniffin' task, $p = 0.003$) associated with soil Mn concentrations, and tremor intensity with hair ($p = 0.01$) and blood Mn ($p = 0.005$), among the adolescents of the impacted area (Lucchini et al., 2012). The elderly subjects residing in the same areas showed similar impairment of motor coordination, hand dexterity and odor identification as the adolescents (Rentschler et al., 2012). The influence of genetic polymorphism was assessed considering the ATP13A2 gene, also known as PARK9, for a protective role in both Parkinson's Diseases and Mn toxicity (Gitler et al., 2009). Polymorphisms rs4920608 and rs2871776 significantly modified the effects of Mn exposure on impaired motor coordination in elderly (p for interaction = 0.03, $p = 0.04$ respectively), also after adjustments for age and gender (Rentschler et al., 2012). In addition, the rs2871776 G allele that was associated with the worst effect of Mn on motor coordination was linked to alteration of a binding site for the transcription factor insulinoma-associated 1 (INSM1). This gene plays an important role in the developing CNS, and especially of olfactory progenitors, as shown in mouse (Rosenbaum et al., 2011) and human embryos (Duggan et al., 2008).

While the accumulation of NPs in brain, whether in the CNS parenchyma or in the vasculature, has been clearly demonstrated, less information is available concerning potential health-related outcome(s) from NP exposure. Elder et al. (2006) showed increases in pro-inflammatory mediators and markers of oxidative stress and immune cell activation (e.g. tumor necrosis factor-α (TNF-α), superoxide dismutase, and GFAP) in rat brain regions where Mn accumulated following Mn oxide NP exposure. These findings suggest that NP exposure resulted in CNS oxidative stress and inflammation.

2.2. Particle exposure, oxidative stress, and neurotoxicity

When reports began emerging in the epidemiological literature suggesting that inhaled ambient particulate-containing air pollution could induce adverse cardiovascular health outcomes (Peters et al., 2000; Pope et al., 1999; Schwartz, 1999), the underlying mechanisms that might explain the observations were unclear. Two plausible mechanisms that were pursued were (1) the release of soluble mediators (inflammatory and/or hormonal) from respiratory tract target cells and (2) the translocation of air pollution constituents out of the lungs to secondary target tissues. Similar mechanisms may serve as plausible explanations for responses of the CNS following pollutant exposure.

The association between oxidative stress, inflammatory processes, and neurodegenerative disease is being currently investigated. For example, a two-fold faster rate of decline in Alzheimer's disease (AD) patients is associated with acute systemic inflammation as determined via increases in serum TNF-α (Holmes et al., 2009). Furthermore, McAlpine et al. (2009) showed that the inhibition of TNF signaling significantly

reduced amyloid beta protein deposition in brain in a mouse model of AD.

Subsequent studies with an oxidative stress-compromised (i.e. ApoE$^{-/-}$) mouse model and their normal C57BL/6 background strain have shed additional light on the role of oxidative stress in particulate matter (PM)-induced neurotoxicity (Veronesi et al., 2005). Veronesi exposed these mice to concentrated ambient particulate matter (CAPs) collected from a state park in Tuxedo, NY. After the 6–7 months exposure, the mice were killed and formalin-perfused. The brains were serially sectioned and immunohistologically stained for tyrosine hydroxylase (TH), a marker for dopaminergic neurons and for GFAP, a marker of astrocytic proliferation. TH-neuronal loss in the substantia nigra was analyzed morphometrically. No significant differences in air or CAPs-exposed wild-type mice were seen. However, a 29% reduction in TH-neuronal loss was shown in the CAPs exposed brains of Apo E$^{-/-}$ mice relative to air-exposed Apo E$^{-/-}$ mice. This established oxidative stress as a predisposing condition for the brain damage associated with particulate matter air pollution (Veronesi et al., 2005).

Since microglia (brain macrophages) are critical to oxidative stress-mediated neurodegeneration (Block et al., 2007), the BV2 cells (immortalized mouse (C57/BL6) microglial cell line) were exposed to samples of CAPs and their cellular and genomic response examined. Exposure of the BV2 microglia to CAPs reduced intracellular levels of ATP and stimulated pro-inflammatory cytokines TNF-α and interleukin-6 after 6 h of exposures. Microarrays on the CAPs exposed BV2 microglia indicated that exposure stimulated genes associated with innate immune pathways (e.g. toll like receptor signaling), oxidative stress-mediated inflammation (e.g. Notch activating pathway for nuclear factor-kB signaling), and multiple pro-apoptotic pathways (Sama et al., 2007). The mechanism(s) underlying PM-CNS toxicity are still unresolved but these and other studies suggest that neurotoxicity in the PM-exposed animals is related to oxidative stress and innate immune pathways.

3. Conclusions

The "nose–brain" interaction as a route of entry for air pollutants is a topic of emerging interest that deserves further research, especially considering the possibility that NP can be transported to target brain areas. The transport of other toxic components of air pollution, such as gases (ozone, carbon monoxide, sulfur oxides, nitrogen oxides), organic compounds (polycyclic aromatic hydrocarbons and endotoxins) and metals (vanadium, nickel, manganese) also need to be considered. In vivo and in vitro evidence supports the epidemiological evidence of neurotoxicity following exposure to air pollution, with an important role played by the olfactory tract. Although mechanisms driving air pollution-induced CNS pathology are poorly understood, new evidence suggests that microglial activation may be a key component, with an important contribution of conditions that predispose the individual to oxidative stress. In summary, although the transport of airborne particles and NP is demonstrated through the olfactory tract, further research must be addressed to explain the mechanisms of toxicity, focusing on the inflammatory changes that are induced by this type of exposure. Early data also suggest that children may be at particular risk from air pollution exposure since childhood and adolescence are crucial periods of brain development associated with dynamic behavioral, cognitive and emotional changes.

Conflict of interest statement

No conflict of interest declared.

Acknowledgments

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