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Developmental neurotoxicity of inhaled ambient ultrafine particle air pollution: Parallels with neuropathological and behavioral features of autism and other neurodevelopmental disorders

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ABSTRACT

Accumulating evidence from both human and animal studies show that brain is a target of air pollution. Multiple epidemiological studies have now linked components of air pollution to diagnosis of autism spectrum disorder (ASD), a linkage with plausibility based on the shared mechanisms of inflammation. Additional plausibility appears to be provided by findings from our studies in mice of exposures from postnatal day (PND) 4–7 and 10–13 (human 3rd trimester equivalent), to concentrated ambient ultrafine (UFP) particles, considered the most reactive component of air pollution, at levels consistent with high traffic areas of major U.S. cities and thus highly relevant to human exposures. These exposures, occurring during a period of marked neuro- and gliogenesis, unexpectedly produced a pattern of developmental neurotoxicity notably similar to multiple hypothesized mechanistic underpinnings of ASD, including its greater impact in males. UFP exposures induced inflammation/microglial activation, reductions in size of the corpus callosum (CC) and associated hypomyelination, aberrant white matter development and/or structural integrity with ventriculomegaly (VM), elevated glutamate and excitatory/inhibitory imbalance, increased amygdala astrocytic activation, and repetitive and impulsive behaviors. Collectively, these findings suggest the human 3rd trimester equivalent as a period of potential vulnerability to neurodevelopmental toxicity to UFP, particularly in males, and point to the possibility that UFP air pollution exposure during periods of rapid neuro- and gliogenesis may be a risk factor not only for ASD, but also for other neurodevelopmental disorders that share features with ASD, such as schizophrenia, attention deficit disorder, and periventricular leukomalacia.

1. Introduction

1.1. Air pollution exposure

Air pollution is a complex mixture of particles, gases, trace metals and adsorbed organic contaminants. Particle sizes range from coarse (2.5–10 μm) to fine (<2.5 μm) to ultrafine (UFP, <100 nm or 0.1 μm). Although not a significant component by mass, UFPs achieve orders of magnitude higher particle number concentrations and thus have more extensive surface areas that can adsorb toxic air pollutants (oxidant gases such as ozone, NO_X, organic compounds, transition metals) per unit mass. Ambient UFPs arise primarily from combustion of fossil fuels (i.e., motor vehicle traffic) (Lippmann et al., 2013). Levels of up to 50% of inhaled UFP are deposited in pulmonary alveolar regions of lung from where they can traverse the alveolocapillary barrier to access pulmonary interstitium and cross endothelial cells into blood circulation and subsequently impact other organs, including heart and brain, and thereby lead to more serious health consequences, which is why UFPs are considered among the most reactive elements of air pollution (Oberdorster et al., 1994; Brown et al., 2001). Deposition can also occur in the nasal cavity allowing translocation to brain (Elder et al., 2006; Hunter and Dey, 1998; Lewis et al., 2005).

Air pollution is a worldwide environmental health problem, to which exposures begin at gestation, and are cumulative over the lifespan. While air quality standards have clearly improved in many countries, even as of 2012, the U.S. Census Bureau estimated that approximately 142 million U.S. residents live in counties where regulated levels of various air pollutants exceeded

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standards set by the U.S. Environmental Protection Agency (EPA). Further, ongoing urbanization trends and expanding road traffic in many areas of the world are predicted to further increase population exposures to UFP air pollution (Kumar et al., 2014). Currently, the U.S. EPA sets regulatory standards for levels of PM_{10} and $PM_{2.5}$. In addition to the fact that it represents a significant technological challenge, regulation of UFPs is said to await additional evidence of their toxicity. Considered in a global context, new studies ascribe 3.3 million premature deaths per year to outdoor air pollution (Lelieveld et al., 2015), and as of 2013, air pollution was listed as the 12th leading global risk factor for disability adjusted life year reductions (Forouzanfar et al., 2013).

1.2. Air pollution and brain

While the focus of air pollution research has long centered on cardiovascular and pulmonary systems, evidence now shows that air pollution also adversely impacts brain, and does so by mechanisms similar to those operative in lung, i.e., via inflammation and oxidative stress (Block and Calderon-Garciduenas., 2009). In children, various components of air pollution have been associated with impaired cognitive abilities (Harris et al., 2015; Suglia et al., 2008), deficits in attention-related behaviors (Chiu et al., 2013; Newman et al., 2013; Siddique et al., 2011), reduced mental development index and IQ scores (Perera et al., 2009), symptoms of anxiety/depression (Perera et al., 2006, 2012), decreased nonverbal reasoning ability (Edwards et al., 2010) and delayed psychomotor development (Guxens et al., 2015). Animal models confirm behavioral and CNS toxicity in response to gestational, postnatal and adult exposures to air pollution and/or its components (Block and Calderon-Garciduenas., 2009), including findings of depressive-like behaviors, impaired spatial learning and memory, reduced apical dendritic spine density and dendritic branching in hippocampus (Fonken et al., 2011), reduced numbers of hippocampal neuronal GluA1glutamate receptor subunits and altered neuronal differentiation of cortical neurons (Davis et al., 2013) and altered locomotor activity and levels of brain catecholamines (Suzuki et al., 2010).

Interestingly, the combined presentation of many of these specific impairments is consistent with the multifaceted nature of Autism Spectrum disorders (ASD) that has now reached a worldwide prevalence as high as 1–2% (Lai et al., 2014; Elsabbagh et al., 2012), with a clear bias towards males being more affected (Elsabbagh et al., 2012; Frazier et al., 2014; Van Wijngaarden-Cremers et al., 2014; Werling and Geschwind, 2013). Diagnosis of ASD relies on the identification of impairments of core behavioral features like social deficits, impaired communication, and repetitive/perseverative behaviors early in life. In fact, while the etiology of this highly intractable and extremely heterogeneous neurodevelopmental disorder remains unknown, numerous epidemiological studies of populations from different states within the U.S. and different countries now report associations of ASD diagnosis with components of air pollution, including residential distance from the freeway (Volk et al., 2011), ambient levels of metals (Roberts et al., 2013; Windham et al., 2006), aromatic solvents (Roberts et al., 2013; Windham et al., 2006; Kalkbrenner et al., 2010), styrene and chromium (Talbott et al., 2015), gases (Becerra et al., 2013; Jung et al., 2013; Volk et al., 2013), diesel exhaust (Roberts et al., 2013; Windham et al., 2006) and particulate matter ($PM_{2.5}$ and/or PM_{10}) (Becerra et al., 2013; Jung et al., 2013; Volk et al., 2013; Kalkbrenner et al., 2015; Raz et al., 2014). Some report increased odds ratios for ASD diagnoses in relation to levels of $PM_{2.5}$ (Jung et al., 2013; Volk et al., 2013; Raz et al., 2014), which includes UFPs, and/or NO_2, a surrogate for UFP (Beckerman et al., 2008). The one exception to such findings was a recent study that combined data across 4 cohorts, but, unfortunately, each cohort used different measures of autistic traits, effectively limiting power and comparability (Guxens et al., 2015).

Underlying reasons for an association of air pollution with ASD are not known, but several lines of evidence suggest that air pollution-induced inflammatory mechanisms during development could play a key role (Melillo and Leisman, 2009; Noriega and Savelkoul, 2014; Meldrum et al., 2013; Depino, 2013; Onore et al., 2012). For example, markers of inflammation are elevated in amniotic fluid (Abdallah et al., 2013), and through life (Gesundheit et al., 2013; Emanuele et al., 2010; Vargas et al., 2005) in ASD, consistent with a chronic inflammatory state in both periphery (Depino , 2013; Emanuele et al., 2010; El-Ansary and Al-Ayadhi., 2012; Naik et al., 2011) and brain (Vargas et al., 2005; Rose et al., 2012; Wei et al., 2011; Li et al., 2009). Increased cytokine levels, a consequence of inflammation, associate with severity of ASD diagnostic features (Ashwood et al., 2011). Correspondingly, multiple components of air pollution including diesel exhaust, fine as well as UFP, and toxicants adsorbed to particles, such as endotoxin (LPS), also produce inflammatory changes in brain (Bolton et al., 2012; Campbell et al., 2009; Hartz et al., 2008; Levesque et al., 2011a,b; Morgan et al., 2011; van Berlo et al., 2010).

Mechanistically, it has been proposed that air pollution-associated CNS pathology reflects interactive pathways that include systemic inflammation, as mediated e.g., by vagal nerve afferents (Oberdorster et al., 2004, 2009), in conjunction with direct effects of particulate matter on brain tissue (Block and Calderon-Garciduenas., 2009). Perhaps not surprisingly, levels of asthma, a disease associated with air pollution-induced inflammation (Wendt et al., 2014; Evans et al., 2014; MacIntyre et al., 2013; Gonzalez-Barcala et al., 2013), are substantially higher in children with ASD, consistent with common inflammatory mechanisms in lung and brain. In a nationally representative sample of 77,951 children, asthma was 35% more common in those with ASD after controlling for age, gender, body mass index, race, brain injury, secondhand smoke and socioeconomic status (Kotey et al., 2014); further the prevalence of both ASD and asthma has been increasing (Becker, 2007; Becker and Schultz, 2010).

Potential mechanistic links may also result from alterations in glutamate. Hyperglutamate function has been hypothesized to underlie ASD (Jacob et al., 2011), and air pollution impacts brain glutamate function (Davis et al., 2013). Both developing and adult microglia have functional glutamate receptors and microglial activation can engender glutamate release (Murugan et al., 2013; Takeuchi et al., 2005). Furthermore, glutamate release can also activate microglia, leading to a vicious cycle (Biber et al., 2007), and a potential mechanism of chronic inflammation.

This paper summarizes the results of a series of studies, including previously published findings and new data describing the impact of exposures of mice to concentrated ambient particles (CAPS) in the *ultrafine* particle (UFP) range that collectively appear to provide biological plausibility for an association between air pollution and ASD. As presented below, exposures of mice to real time, road inlet ambient concentrated (10–20*x*) UFP from postnatal days (PND) 4–7 and 10–13 (equivalent to human 3rd trimester (Clancy et al., 2007a,b; Rice and Barone, 2000), and a period of marked neuro- and gliogenesis (Bandeira et al., 2009), unexpectedly produced a pattern of developmental neurotoxicity notably similar to multiple hypothesized mechanistic underpinnings of ASD and other neurodevelopmental disabilities. Our exposure paradigm is highly relevant to human exposures as UFPs, considered the most reactive component of air pollution, occurred at levels consistent with high traffic areas of major U.S. cities.

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