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Developmental neurotoxicity of traffic-related air pollution: focus on autism

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Abstract

Purpose of Review—Epidemiological and animal studies suggest that air pollution may negatively affect the central nervous system (CNS) and contribute to CNS diseases. Traffic-related air pollution is a major contributor to global air pollution, and diesel exhaust (DE) is its most important component.

Recent findings—Several studies suggest that young individuals may be particularly susceptible to air pollution-induced neurotoxicity, and that perinatal exposure may cause or contribute to developmental disabilities and behavioral abnormalities. In particular, a number of recent studies have found associations between exposures to traffic-related air pollution and autism spectrum disorders (ASD), which are characterized by impairment in socialization and in communication, and by the presence of repetitive and unusual behaviors. The cause(s) of ASD are unknown, and while it may have a hereditary component, environmental factors are increasingly suspected as playing a pivotal role in its etiology, particularly in genetically susceptible individuals.

Summary—Autistic children present higher levels of neuroinflammation and systemic inflammation, which are also hallmarks of exposure to traffic-related air pollution. Gene-environment interactions may play a relevant role in determining individual susceptibility to air pollution developmental neurotoxicity. Given the worldwide presence of elevated air pollution, studies on its effects and mechanisms on the developing brain, genetic susceptibility, role in neurodevelopmental disorders, and possible therapeutic interventions, are certainly warranted.

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Compliance with ethics guidelines

Conflict of interest

Lucio G. Costa, Yu-Chi Chang, and Toby B. Cole declare that they have no conflicts of interest.

Human and Animal Rights and Informed Consent

This review article does not contain any studies with human or animal subjects performed by any of the authors. Studies with animals by the authors and reported elsewhere are referred to. These studies were approved by the Institutional Animal Review Board.

Keywords

Traffic-related air pollution; diesel exhaust; neuro-inflammation; autism spectrum disorders; reelin; gene-environment interactions

Introduction

Air pollution is a mixture of several components, including gases, organic compounds, metals, and ambient particulate matter (PM); the latter is believed to be the most widespread threat, and has been heavily implicated in disease [1, 2]. The populations of many countries, particularly in South and East Asia, are often exposed to relatively high levels of PM ($100 \mu\text{g}/\text{m}^3$) [3, 4]. PM is broadly characterized by aerodynamic diameter (e.g. PM_{10} , equivalent to $<10 \mu\text{m}$ in diameter). Traffic-related air pollution is a major contributor to global air pollution, and diesel exhaust (DE) is its most important component [5]. DE contains more than 40 toxic air pollutants, and is a major constituent of ambient PM, particularly of fine ($\text{PM}_{2.5}$) and ultrafine (UFPM; $<100 \text{ nm}$) PM [6]. DE exposure is often utilized as a measure of traffic-related air pollution. The association between air pollution and morbidity and mortality caused by respiratory and cardiovascular diseases is well established [7, 8], and oxidative stress and inflammation are believed to be the most relevant contributors to such effects [9].

In recent years evidence has been accumulating from human epidemiological and animal studies which indicates that air pollution may negatively affect the central nervous system (CNS) and contribute to CNS diseases [10–15]. $\text{PM}_{2.5}$ and UFPM are of much concern, as these particles can enter the circulation and distribute to various organs, including the brain [12, 16], in addition to gaining direct access to the brain through the nasal olfactory mucosa [17–20]. Decreased cognitive function, olfactory dysfunction, auditory deficits, depressive symptoms and other adverse neuropsychological effects have also been reported [21–26]. Post-mortem investigations in highly exposed individuals have revealed increased markers of neuroinflammation and of neurodegenerative pathologies [24, 27–29]. Animal studies corroborate the human observations [2, 30]. For example, dogs exposed to heavy air pollution presented evidence of chronic inflammation and neurodegeneration in various brain regions [10, 31], and mice exposed to traffic in a highway tunnel had higher levels of pro-inflammatory cytokines in brain [32]. Controlled exposure to DE has been reported to alter motor activity, spatial learning and memory, and novel object recognition ability in mice, and to alter emotional behavior and learning capability in rats [33, 34]. Prominent effects of DE exposure in the CNS are oxidative stress and neuro-inflammation [35–40].

Developmental neurotoxicity of air pollution

Epidemiological and animal studies suggest that young individuals may be particularly susceptible to air pollution-induced neurotoxicity [22, 24, 26–28, 41–45]. Human studies have revealed a series of biochemical and behavioral alterations in children exposed pre- and/or post-natally to elevated air pollution. In addition, developmental exposure to air pollution, particularly traffic related air pollution, has been suggested to play a role as an etiological factor in autism spectrum disorders (see following sections).

A series of studies in Mexico City have revealed elevated levels of neuroinflammatory markers in brain of children exposed to high air pollution, as well as cognitive deficits [24, 27, 42, 46]. Saenen et al. [47] found a decreased expression of genes associated with the brain-derived growth factor signaling pathway in placenta upon exposure to PM_{2.5}. Newman et al. [48] reported hyperactivity in 7-year old children associated with early life exposure to traffic-related air pollution. In six European cohorts, exposure to air pollution during pregnancy was found to be associated with delayed psychomotor development [43]. Similar results were found in a study in Japan, in which air pollution exposure during gestation was associated with delays in developmental milestones in children at both 2.5 and 5.5 years of age [49]. Additional studies reported that exposure to traffic-related air pollution was inversely associated with sustained attention in adolescents [50], and to lower cognitive development in primary school children [51]. The latter was confirmed in another study in Spain, in which developmental exposure to PM_{2.5} was associated with a 11–30% reduction in cognitive development [52]. In a population of children in Eastern Massachusetts, mid-childhood exposure to air pollution, particularly to black carbon, was reported to be associated with diminished executive functions at 6–10 years of age [53]. Chiu et al. [53] reported that pre-natal exposure to air pollution was associated with a number of behavioral alterations in children, mostly in boys. In particular, exposure to PM_{2.5} in gestational weeks 31–36 was associated with lower IQ, while earlier exposures (weeks 20–26) were associated with lower attention. Deficits in reaction time and memory were also found [54]. In a recent review Xu et al. [55] identified a total of 41 human studies which examined the potential effects of ambient or traffic-related air pollution on children (including those specifically investigating autism). They concluded that “evidence suggests that prenatal exposure to air pollutants may have impacts of child neurodevelopment regardless of different study designs, study populations, air pollution exposure assessments, and outcome measurements” [55].

Experimental studies also indicate that developmental exposure to DE may cause neurotoxicity [56]. *In utero* exposure to high levels of DE (1.0 mg/m³) caused alterations in motor activity, motor coordination and impulsive behavior, as well as changes in neurotransmitters, in male offspring [34, 57, 58]. Depression-like responses were found in mice exposed prenatally to urban air nanoparticles at somewhat lower concentrations (350 µg/m³) [59]. Additional studies have shown that developmental DE exposure of mice alters motor activity, spatial learning and memory, and novel object recognition ability, and causes changes in gene expression, neuro-inflammation, and oxidative damage [33, 60–64]. Prenatal exposure of mice to a low level of DE (90 µg/m³) has been found to enhance territorial aggression induced by social isolation in male mice [65]. Early postnatal exposure of mice to concentrated ambient PM was reported to cause various behavioral changes, including long-term impairment of short term memory, and impulsivity-like behavior [66, 67]. Additional human and animal studies have focused on the potential effects of developmental air pollutant exposure on autism-like behaviors and of their potential etiological role in autism, and are discussed in a following section.

Developmental exposure to air pollution and autism spectrum disorders

Autism is a neurodevelopmental disorder characterized by marked reduction of social and communicative skills, and by the presence of stereotyped behaviors [68]. The term autism spectrum disorders (ASD) is usually utilized to include autism and a range of similar disorders, such as Asperger's syndrome. The symptoms of ASD are typically present before the age of three, and are often accompanied by abnormalities of cognitive functioning, learning, attention, and sensory processing [68]. The incidence of ASD appears to have increased in the past few decades, and it is now estimated at about 7–9/1000, though certain studies have identified up to 27/1000 children affected by ASD [69, 70]. ASD is more common in males than in females [71], and represents an important societal problem, as the economic burden of caring for an individual with ASD and intellectual disability during his/her lifespan has been estimated at \$2.4 million [72]. Children with ASD present a number of morphological abnormalities in the brain [68, 73, 74], and alterations in certain neurotransmitter systems [75]. They also have higher levels of oxidative stress [76–78], as well as neuroinflammation and increased systemic inflammation [79–83].

The etiological basis of ASD is unknown, and susceptibility is attributable to both genetic and environmental factors [68, 84–88]. Several candidate susceptibility genes for ASD have been identified, but no single anomaly appears to predominate, and the total fraction of ASD attributable to genetic inheritance may be only about 30–50% [85, 89]. DNA methylation is also altered in the autistic brain, suggesting that epigenetic dysregulation may also contribute to ASD [90, 91]. It is thus apparent that ASD likely results from the complex interactions between genes conferring vulnerability and diverse environmental factors. In addition to air pollution (particularly traffic-related), which is discussed in the next section, chemicals studied in this regard include metals (e.g. mercury, lead), pesticides (e.g. organophosphates) and other industrial chemicals (e.g. polybrominated diphenyl ethers, organochlorine compounds) [87, 88, 92, 93]. Perhaps the strongest association between an environmental factor and ASD has been found with maternal infection [94]. Studies in humans and in various animal species have indeed evidenced that maternal immune activation (MIA), due to viral or bacterial infection, increases neuroinflammation in the placenta and in the fetal brain, leading to offspring that display ASD-like behaviors [95–98]. As discussed in a further section, several effects seen in MIA are also found upon developmental exposure to air pollution.

Traffic-related air pollution and ASD

Several studies have found associations between exposures to traffic-related air pollution and ASD [2, 99]. Two studies in California by Volk et al. [100, 101] found that residential proximity to freeways and gestational and early-life exposure to traffic-related air pollution were associated with autism (OR=1.86; 95% CI=1.04–3.45). Similar results were obtained in another epidemiological study in California [102], and in another one, part of the Nurses' Health Study II, in which perinatal DE exposure was significantly associated with ASD, particularly in boys [103]. Two further studies in Taiwan [104] and in Pennsylvania [105] also reported of an increased risk of ASD associated with PM and air pollution exposure, while a study by Guxens et al. [106] in four European cohorts found no associations. An

additional study in two cohorts in North Carolina and California reported an association between PM exposure and ASD, particularly when exposure occurred in the third trimester of pregnancy [107]. The higher susceptibility of third trimester exposure was also evidenced by a study of Raz et al. [108] in the Nurses' Health Study II cohort.

The few available animal studies are in agreement with the human observations [2, 30]. Prenatal exposure to DE has been shown to disrupt DNA methylation in the brain [109]. Prenatal and early life exposure of mice to DE is associated with a number of behaviors similar to those present in humans with ASD, including higher levels of motor activity, elevated levels of self-grooming, and increased rearing [110]. Postnatal exposure, on postnatal days (PND) 4–7 and 10–13, to concentrated ambient ultrafine particles caused persistent glial cell (astrocytes and microglia) activation, and ventriculomegaly (lateral ventricular dilation), which occurred preferentially in male mice [67, 111]. Brain region- and sex-dependent alterations in cytokines and neurotransmitters were also found in exposed male and female mice [111]. Using the same exposure protocol these investigators also reported a decreased corpus callosum in both male and female mice, and an increase of glutamate levels, with an excitatory/inhibitory imbalance [112]. Chang et al. (in preparation) found that perinatal exposure of mice to DE at environmentally relevant concentrations [250–300 µg/m³, from gestational day (GD) 0 to PND 21] caused significant behavioral alterations relevant to ASD, in the domains of persistent/repetitive behaviors, communication, and social interactions. Interestingly, the effects of developmental DE exposure were more robust if exposure occurred in both the pre-natal (GD 0 - birth) and post-natal (PND 1–21) periods. Human studies indicated that the association between air pollution and ASD is stronger when exposure occurs in the third trimester of pregnancy [107, 108, 113, 114]. Due to different rates of brain development, the third trimester of pregnancy in humans is equivalent to the first few postnatal weeks in mice and rats [115, 116]. Animal studies, which report robust effects when exposure occurred or continued postnatally [66, 67, 110; Chang et al. in preparation], are thus in agreement with human observations.

Possible mechanisms of developmental neurotoxicity of traffic related air pollution

Currently, the most prominent reported effects of air pollution on the CNS are related to microglia activation with ensuing oxidative stress and neuroinflammation. Such effects have been found *in vivo* [35–39], and have been reproduced *in vitro* [117, 118]. For example, in the latter study [118] it was found that diesel exhaust particles activate microglia; microglia-generated oxidant species and pro-inflammatory cytokines such as IL-6 cause neuronal toxicity, which can be prevented by inhibiting microglia activation.

This chain of events may explain many of the observed effects seen in brain of rodents following developmental air pollution exposure. For example, microglia-generated pro-inflammatory cytokines could lead to the observed hypomyelination and ventriculomegaly via toxicity to oligodendrocytes [112]. Closely related to ASD is also the hypothesis of a possible impairment by DE of the reelin signaling system (see Fig. 1). Reelin is a signaling

glycoprotein, secreted in the marginal zone of the developing cerebral cortex by Cajal-Retzius cells [119], which plays a most relevant role in neuronal migration and establishment of neuronal polarity [120–123]. In the adult nervous system, reelin is expressed in GABAergic interneurons in the cortex and the hippocampus, where it modulates learning and memory processes, and its reduction may contribute to Alzheimer's disease [124]. The canonical reelin signaling pathway is activated upon binding of reelin to VLDL receptor and ApoE receptor 2, which triggers tyrosine phosphorylation of the intracellular adaptor protein disabled-1 (Dab1). Phosphorylated Dab1 then activates a kinase cascade involving PI-3 kinase, LIM kinase-1, and several others [123]. Such complex networks of signaling pathways mediate the ultimate effects of reelin on neuronal migration and polarity in the developing brain. Strong evidence exists for an involvement of reelin in ASD. First, reelin expression is significantly decreased in brain from ASD subjects [121, 125]. Second, the reelin gene, which maps at chromosome 7q22, is affected in several autistic pedigrees [126–129]. Third, the methylation pattern at the reelin gene promoter is different in ASD and control post-mortem brains [130]. Fourth, mice lacking the C-terminal region of reelin exhibit behavioral abnormalities related to ASD [131]; Fifth, the reeler (*rl^{-/-}*) mouse, a spontaneously arising mutant mouse, displays several ASD-like morphological and behavioral traits [132, 133]. Sixth, cortical disorganization has been reported in reelin-deficient mice and in ASD patients [74, 134]. Seventh, dysregulation of reelin-driven cortical neuron migration is present in ASD [133]. In addition to all this, MIA which leads to offspring that display neuro-inflammation and ASD-like behaviors [95, 96], has been shown to decrease levels of reelin protein and mRNA in brain of offspring [135–138]. The notion that oxidative stress and neuroinflammation may play an important role in modulating reelin expression is also supported by studies showing that N-acetylcysteine completely prevents lipopolysaccharide (LPS)-induced decreases of reelin [137]. In our laboratory we have found that developmental DE exposure (250–300 µg/m³ from GD0 to PND21) causes neuroinflammation (as evidenced by an increase in IL-6 mRNA) and a decrease of reelin expression (Chang et al. unpublished results).

Additional mechanistic hypotheses may and should be formulated with regard to possible effects of developmental air pollution exposure on the observed excitatory/inhibitory imbalance, which is believed to be relevant in ASD [139]. While such imbalance may be due to a reduced GABAergic action or to an increased glutamatergic one, recent evidence suggests that in individuals with ASD the deficit lies in a reduced GABAergic action [140]. The “reelin hypothesis” discussed above may provide at least a partial mechanistic explanation even in this case. By inducing neuroinflammation, and specifically by increasing levels of IL-6, air pollution would also increase expression of DNA methyltransferase-1 (DNMT1) via the JAK/STAT pathway [141]. DNMT1, which in turn modulates the expression of reelin [142, 143], has been found to be increased upon developmental DE exposure (Chang et al. unpublished results). Since DNMT1 also decreases the expression of GAD 67 (glutamic acid decarboxylase 67), a marker of inhibitory GABAergic interneurons [143], this decrease would diminish inhibitory GABAergic neurotransmission, thereby disrupting the balance of excitation/inhibition, as found in ASD and in mouse models of ASD [144]. Of interest is that also maternal immune activation causes a decrease of GAD67 [145].

Possible gene-environment interactions in the developmental neurotoxicity of traffic related air pollution

Animal models that resemble core human autistic symptoms may be useful for studying the etiology and molecular pathogenesis of autism, and for discovering gene-environment interactions [146]. Various strains of mice have been identified that display at least some behavioral traits relevant to ASD, either carrying specific genetic mutations [144, 147], or others whose genetic traits have not been fully characterized, such as the BTBR mouse [148, 149]. However, the marked alterations already present in these mice may represent a “ceiling” effect, and these strains may not be amenable to investigate gene-environment interactions. Nevertheless, De Felice et al. [150, 151] investigated the effects of the organophosphorus insecticide chlorpyrifos in BTBR mice exposed *in utero*. They found that the effects of chlorpyrifos on oxidative stress and on behavioral maturation were enhanced in BTBR mice compared to C57 mice. These findings suggest that these mice may also be amenable for studying the developmental neurotoxicity of air pollution.

Another interesting transgenic model to investigate potential gene-environment interactions related to developmental DE exposure and ASD, may be the heterozygote reeler mouse ($rl^{+/-}$). In contrast to the reeler mouse ($rl^{-/-}$) in which the absence of reelin causes severe disorganization of brain development and severe behavioral effects [132], the $rl^{+/-}$ mouse displays only moderate behavioral abnormalities [152, 153]. The applicability of this model has been shown by the finding that developmental exposure of $rl^{+/-}$ mice to 6 ppm methylmercury increases ASD-like behaviors, particularly in male animals, compared to $rl^{+/+}$ mice [154]. The hypothesis discussed in the previous section involving a primary role for reelin in the developmental neurotoxicity of air pollution, would be in tune with a potential gene-environment interaction in $rl^{+/-}$ mice.

As oxidative stress and neuroinflammation are preponderant responses to DE exposure [35, 36, 38, 39], another potential interesting model is represented by the *Gclm* mouse, which lacks the modifier subunit of glutamate-cysteine ligase, the first and rate-limiting enzyme in the synthesis of glutathione (GSH), a main player in cellular defense against oxidative stress. *Gclm*^{-/-} mice have very low levels of GSH in all tissues including the brain [155], though they may up-regulate other antioxidant pathways; in contrast, *Gclm*^{+/-} mice have only moderate reductions in GSH, but may not up-regulate alternate defense pathways. In addition, *Gclm*^{+/-} mice may more closely resemble a very common human polymorphism of *Gclm* [156]. *Gclm*^{+/-} mice have been shown to be most sensitive to oxidative stress and neuro-inflammation induced by acute DE exposure (250–300 µg/m³ for 6 h) [30, 39]. This finding confirms a previous observation of enhanced lung inflammation in *Gclm*^{+/-} mice upon exposure to DE compared to wild type mice [157]. Of great relevance is also the finding that in brain of subjects with ASD, there is a 37% decrease of GCLM protein level, and a 38% decrease in GCL activity [158], which is in agreement with the reported reduced levels of GSH [76]. Thus, the proposed transgenic model (*Gclm*^{+/-} mice) would be highly relevant to study gene-environment interactions related to developmental exposure to air pollution and ASD.

Conclusion and research needs

While several chemicals present in the environment or in the diet have been considered and studied for potential developmental neurotoxicity, little had been done until recently in this regard for chemicals present in the air. Yet, the air we breathe seems a logical potential source of exposure for chemicals which may exert neurotoxicity or developmental neurotoxicity. Though attention has been limited for several decades only to effects on the respiratory system, and more recently on the cardiovascular system, evidence has been accumulating during the past several years providing strong support to the notion that exposure to high levels of air pollution, very common in many cities all around the world, is associated with damage to the CNS. Human and animal studies have evidenced a series of common adverse effects of air pollution (particularly traffic-related), with oxidative stress and neuro-inflammation emerging as the hallmark biochemical effects, and clinical manifestations which included a variety of behavioral alterations.

As the developing nervous system is particularly sensitive to toxic insult [159], the issue of developmental neurotoxicity of air pollution is especially relevant. Particularly troublesome is the suggestion that air pollution may contribute to the etiopathology of neurodevelopmental diseases whose incidence seems to be increasing in the global populations. This review has focused on ASD, which have been the most studied in this regard, but other disorders such as early onset schizophrenia, or attention deficit hyperactivity disorder, also need to be considered.

Measures to decrease emissions leading to poor air quality are the obvious first choice to pursue in order to protect human health. However, further studies aimed at better characterizing the effects of air pollution on the CNS, its underlying mechanisms, and its role in the etiology of neurodevelopmental diseases are certainly warranted. In particular, the possibility that sexes may be differentially affected by air pollution, with males being more susceptible, needs to be further investigated, in light of the higher incidence of neurodevelopmental disorders (e.g. ASD) in males [71]. In addition, gene-environment interactions still need to be investigated in the context of exposure to high air pollution and effects on the CNS, as developmental abnormalities are likely to be manifest only or especially in susceptible individuals. In this respect, there is the need for experimental studies utilizing transgenic animal models of certain neurodevelopmental disorders (e.g. the reelin heterozygote mouse for ASD) or other transgenic animals addressing specific mechanistic hypotheses (e.g. the *Gclm*^{+/-} mouse). Markers of genetic susceptibility should also be incorporated in human epidemiological studies, something that has been missing so far. Last but not least, these studies should provide important novel information for therapeutic interventions involving for examples, anti-inflammatory and/or anti-oxidant compounds, drugs that inhibit microglia activation, or others that facilitate GABAergic neurotransmission.

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Fig. 1.
Scheme of a proposed mechanism of developmental effects of DE involving disruption of the reelin pathway (see text for details).