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Perinatal exposure to concentrated ambient particulates results in autism-like behavioral deficits in adult mice

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ABSTRACT

Exposure to fine ambient particulates ($PM_{2.5}$) during gestation or neonatally has potent neurotoxic effects. While biological and behavioral data indicate a vulnerability to environmental pollutants across distinct neurodevelopmental windows, the behavioral consequences following exposure across the entire developmental period remain unknown. Moreover, several epidemiological studies support a link between developmental exposure to air pollution and an increased risk of later receiving a diagnosis of autism spectrum disorders (ASD), a neurodevelopmental disorder that persists throughout life. In the current study we sought to determine whether perinatal exposure to $PM_{2.5}$ would reduce sociability and increase repetitive deficits in mice, two hallmark characteristics of ASD. Pregnant female $B_6C_3F_1$ mice were exposed daily to concentrated ambient $PM_{2.5}$ (CAPs) (135.8 $\mu g/m^3$) or filtered air (3.1 $\mu g/m^3$) throughout gestation followed by additional exposures to both dams and their litters from days 2–10 postpartum. Adult offspring were subsequently assessed for social and repetitive behaviors at 20 weeks of age. Daily perinatal exposure to CAPs significantly decreased sociability in male and female mice as measured by the social approach task; however, reductions in reciprocal social interaction and increased grooming behavior were only present in male offspring exposed to CAPs. These findings demonstrate that exposure to particulate air pollutants throughout early neurodevelopment induces long lasting behavioral deficits in a sex-dependent manner and may be an underlying cause of neurodevelopmental disorders such as ASD.

1. Introduction

Autism spectrum disorders (ASD) are a class of neurodevelopmental disorders characterized by social-communication deficits and restricted/repetitive patterns of behavior (American Psychiatric Association, 2013) affecting an estimated 1 in 42 boys and 1 in 189 girls in the United States alone (Christensen and Baio, 2016). Although ASD is diagnosed during childhood, deficits persist throughout life and ASD-associated costs necessary for adult services are greater than those for children (Buescher et al., 2014), making it essential to understand ASD across all stages of the disorder. While the etiology of ASD remains unknown, epidemiological studies suggest that environmental factors like maternal exposure to air pollutants (Volk et al., 2011; Raz et al., 2015) contribute to ASD susceptibility.

Air pollution is composed of gases, metals, organic material, and ambient particulate matter (PM) (Costa et al., 2014). Fine PM is thought to be the most dangerous component of air pollution, causing neuroinflammation and oxidative damage (Block and Calderón-Garcidueñas, 2009). Decreasing PM size corresponds with increasing severity of injury due to the ease with which smaller particles can enter tissues. PM smaller than 2.5 μm in diameter ($PM_{2.5}$), such as that found in diesel exhaust, accumulates near highways in major cities in the United States and other countries worldwide (Costa et al., 2014, 2017). In fact, epidemiological findings have linked residential proximity to a major highway during pregnancy with an increased childhood risk of an ASD diagnosis (Volk et al., 2011; Raz et al., 2015). More recently, a

Abbreviations: ASD, autism spectrum disorder; CAPs, concentrated ambient particulates; DE, diesel exhaust; FA, filtered air; PM, particulate matter.

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nation-wide case-control analysis across the continental United States revealed higher maternal exposure to $PM_{2.5}$ throughout gestation was associated with greater odds of a child having ASD (Raz et al., 2015). Additionally, although the average $PM_{2.5}$ exposure in the United States is below the World Health Organization's recommended annual mean level of 10 $\mu g/m^3$, many developing nations worldwide are well above this advised limit (WHO, 2016), potentially creating an at-risk environment for pregnant women. While these population-based studies are compelling, further non-human studies are necessary to demonstrate a direct causal relationship between air pollution PM and ASD.

Mouse models of *in utero* or neonatal pollutant exposure show altered neurotransmitter levels, neuroinflammation, impaired hippocampal-dependent memory, and depressive- or anxiety-like behaviors in offspring. For example, gestational exposure to diesel exhaust (DE), a major source of $PM_{2.5}$ pollution, affects neurotransmitter levels in the prefrontal cortex, striatum, hippocampus, hypothalamus, amygdala, cerebellum, and brainstem of offspring, and results in depressive- and anxiety-like behaviors in males specifically (Suzuki et al., 2010; Davis et al., 2013; Bolton et al., 2013; Thirtamara Rajamani et al., 2013; Yokota et al., 2013, 2016). Furthermore, gestational DE exposure combined with maternal stress reduce fear conditioned freezing behavior in adult offspring, indicating long-term impairment in hippocampal-dependent memory (Bolton et al., 2013). Neonatal exposure to fine and ultrafine concentrated ambient PM ($\leq$100 nm) differentially alters cytokine expression and neurotransmitter levels in female and male offspring and increases glial reactivity and hippocampal glutamate in males (Allen et al., 2014, 2017). While these findings highlight the neurotoxic effects of diesel exhaust on brain development, several questions remain regarding the behavioral consequences of $PM_{2.5}$ exposure across gestational and postnatal periods. Specifically, most of the above studies fail to include female offspring in their analysis, and although the prevalence of ASD is greater in males, females are an important population that need equal consideration. Furthermore, no study to date has investigated the effects of developmental air pollution exposure on social and repetitive behaviors, core features of ASD and other neurodevelopmental disorders. Finally, experimental models of gestational or postnatal pollutant exposure focus on outcomes in juvenile and adolescent mice, but none have looked at the long term social behavioral consequences persisting into adulthood, which is essential when modeling a lifelong disorder. Therefore, a more complete understanding of the behavioral and developmental consequences resulting from perinatal pollutant exposure will help elucidate potential mechanisms underlying the etiologies of neurodevelopmental disorders such as ASD that are characterized by similar behavioral deficits.

The current study assessed whether perinatal exposure to concentrated $PM_{2.5}$ (CAPs, 135.8 $\mu g/m^3$) alters well-validated measures of social and restricted/repetitive behaviors in adult male and female B_6C_3 hybrid mice (B6 $\times$ C3H). Specifically, social approach and reciprocal social interaction tasks allow for detection of broad and discrete social interactions, respectively, while increases in time spent self-grooming are often used to model the repetitive self-directed behaviors in individuals with ASD (Mason, 1991; Kelley, 2001; Crawley, 2007). Given that pollutant exposure affects neural circuitry involved in social cognition and repetitive behaviors (Adolphs, 2001; Kalueff et al., 2016), we hypothesized that early life CAPs exposure would decrease sociability and increase grooming behavior, and that this effect would be exacerbated in male compared to female offspring. To test this, pregnant dams were exposed to CAPs or filtered air (FA) throughout gestation and pups were exposed during lactation until postnatal day (P)10 to parallel the third trimester in human pregnancies (Dobbing and Sands, 1979; Romijn et al., 1991). Adult offspring of both sexes were then assessed for ASD-like behavioral deficits using social approach, reciprocal social interaction, and grooming behavioral tasks.

2. Materials and methods

2.1. Animals

Male and female $B_6C_3F_1$ mice (Jackson Laboratory, Bar Harbor, Maine, USA) were purchased and initially housed and maintained for exposure at New York University, Department of Environmental Medicine (Tuxedo, NY) as previously described (Blum et al., 2017). Each animal was housed in polycarbonate cages in temperature (20–23° C) and humidity (~55% RH) controlled rooms on a 12 h light/dark cycle, and food and water were provided *ad libitum*. Females were housed in pairs and males individually for about 10 days. Upon the 2nd proestrus, one male and one female in proestrus were housed together in pairs. Seminal plugs were then checked 12–18 h after breeding pairs were co-housed (gestational day [G]-0.5), and upon presence of a plug, males were removed and females were separated into two exposure groups: Concentrated Ambient Particulate Matter $\leq$2.5 μm (CAPs) or Filtered Air (FA). Food and water were restricted during exposure. A total of 25 litters were subjected to perinatal exposure (13 FA and 12 CAPs), and a subset of the offspring were raised until adulthood for behavioral analyses, FA n = 26 (13 M/13F), CAPs n = 31 (15 M/16F). Following perinatal CAPs exposure, parturition, and weaning, 1–3 male and female offspring from each litter were rehoused 2–4 mice per cage with sex- and treatment-matched cage mates (littermates when possible) and allowed to acclimate for 8 weeks. During this acclimation period, mice were observed daily for signs of aggression. Two male mice were removed from their cages and excluded from the study following evidence of aggression. Following acclimation, mice were transported in standard plastic cages in a temperature controlled van (World Courier, AmerisourceBergen Corporation) to Mount Holyoke College, South Hadley, MA for behavioral assessment. Upon arrival at Mount Holyoke College, 16-week-old mice were group housed with previous cage mates in standard plastic cages and allowed 4 weeks to acclimate to the facilities prior to behavior testing. Mice were maintained at ambient room temperature on a 12 h light/dark cycle (lights on at 0800 h). Food and water were provided *ad libitum* and all cages were given nestlets for enrichment. All behavioral procedures were performed during the first 4 h of the light cycle, and all procedures were approved by both New York University School of Medicine's and Mount Holyoke College's Institutional Animal Care and Use Committees.

2.2. Concentrated fine-size ambient particulate system

After the presence of a seminal plug, female mice were separated and randomly sorted into one of two exposure groups, either CAPs or HEPA-filtered air (FA). Pregnant females were weighed each morning and exposed via whole body inhalation to either CAPs or FA using a modified Versatile Aerosol Concentration Enrichment System (VACES) developed by Sioutas et al. (1999) and modified by Maciejczyk et al. (2008). Ambient air was passed through an Aerotec 2 cyclone inlet to remove the majority of particles greater than 2.5 μm in diameter and then passed through silica gel and carbon filters to remove excess moisture and organic pollutants. PM aerosol was then chilled in a condenser tube and the remaining concentrated particles were passed over a warmed water bath to restore relative humidity. The concentrated aerosol was then divided into three streams for subsequent use: 27% of the particle flow was directed towards Teflon filters housed in Harvard

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