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Developmental exposure to low level ambient ultrafine particle air pollution and cognitive dysfunction

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ABSTRACT

Developmental exposures to ambient ultrafine particles (UFPs) can produce multiple neuropathological and neurochemical changes that might contribute to persistent alterations in cognitive-type functions. The objective of the current study was to test the hypothesis that developmental UFP exposure produced impairments in learning, memory and impulsive-like behaviors and to determine whether these were selective and thus independent of deficits in other behavioral domains such as motor activity or motivation. Performance on measures of learning (repeated learning), memory (novel object recognition, NOR), impulsive-like behavior (differential reinforcement of low rate (DRL), schedule of reward and delay of reward (DOR)), motor activity (locomotor behavior) and motivation (progressive ratio schedule) were examined in adult mice that had been exposed to concentrated (10–20x) ambient ultrafine particles (CAPS) averaging approximately 45 ug/m^3 particle mass concentrations from postnatal day (PND) 4–7 and 10–13 for 4 h/day. Given the number of behavioral tests, animals were tested in different groups. Results showed male-specific alterations in learning and memory functions (repeated learning, NOR and DRL) specifically during transitions in reinforcement contingencies (changes in rules governing behavior) that did not appear to be related to alterations in locomotor function or motivation. Females did not exhibit cognitive-like deficits at these exposure concentrations, but displayed behaviors consistent with altered motivation, including increases in response rates during repeated learning, significantly increased latencies to respond on the delay of reward paradigm, and reductions in the progressive ratio break point. Consistent with our prior findings, male-specific learning and memory-related deficits were seen and occurred even at relatively low level developmental UFP exposures, while females show alterations in motivational behaviors but not final performance. These findings add to the evidence suggesting the need to regulate UFP levels.

1. Introduction

Air pollution, a complex mixture of particles, gases, trace metals, and adsorbed organic contaminants, has been reported to be the 7th leading global cause of mortality (Forouzanfar et al., 2015). Its adverse effects are considered to derive from its ability to produce inflammation and oxidative stress, as has been most extensively examined in cardiopulmonary systems (Kurt et al., 2016; Mills et al., 2009). Air pollution particulate sizes range from coarse (<10 μm) to fine (<2.5 μm) to ultrafine (UFPs; <0.1 μm). The UFP fraction achieves orders of magnitude higher particle count concentrations and surface area than do larger particle sizes, which allows for greater adsorption of other toxic air pollutants such as oxidant gases (e.g., O_3, NO_x), organic compounds, and transition metals per unit mass. UFPs are deposited in pulmonary alveolar regions of lung from where they can access pulmonary interstitium after traversing the alveolocapillary barrier. UFPs can then cross endothelial cells into blood circulation from where they can subsequently impact multiple organs (Elder and Oberdorster, 2006), leading to serious health consequences. UFPs can also deposit in the nasal cavity and from there can be translocated to brain (Oberdorster et al., 2004). Given their inflammatory properties, UFPs are generally considered among the most reactive elements of air pollution (Oberdorster et al., 1994; Oberdorster, 2000).

Increasingly, it is being recognized that air pollution targets other organs and systems of the body, including the central nervous system (CNS). Of particular concern is the developing brain that must undergo a precisely timed sequence of cell proliferation, differentiation, migration, and maturation.

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Disruption of these ontogenetic sequences can lead to neuropathological and morphological changes in brain that can adversely impact behavioral function. Numerous epidemiological studies have reported associations of various measures of air pollution with diagnosis of autism spectrum disorder, a heterogenous disorder diagnosed on the basis of behavioral deficits (Lai et al., 2014; Elsabbagh et al., 2012) including social deficits and perseveration. Increased odds ratios for autism diagnosis have been associated with traffic-related air pollution (Lai et al., 2014; Allen et al., 2017a), diesel exhaust and particles (Basagana et al., 2016), and air toxics (Elsabbagh et al., 2012). Maternal air pollution exposure increased autism risk based on data from across the U.S (Whitten, 1957), with stronger associations for boys, consistent with the sex bias of autism cases. A study of 49,073 Taiwanese children of both sexes reported that air pollution exposure in the preceding 1–4 years increased autism diagnosis risk (Jung et al., 2013).

Multiple studies now also link air pollution exposure with cognitive deficits and reductions in IQ (Allen et al., 2017a). Such studies have included a prospective cohort showing a decline in the trajectory of cognitive growth in relation to levels of $PM_{2.5}$ in school children over a one-year period (Basagana et al., 2016). Another study links air pollution with diagnosis of attention deficit disorder (Siddique et al., 2011), a disorder that can also impact executive and cognitive functions.

In our prior studies of developmental exposures of mice to concentrated ambient ultrafine particles (CAPS), we have observed behavioral, neurochemical and neuropathological deficits particularly in male mice (Allen et al., 2013, 2015). These included deficits in a waiting for reward behavioral paradigm (Allen et al., 2013) that were suggestive of impulsivity, another facet of behavior that could contribute to learning, memory or attention deficits. The current study sought to extend those prior efforts to specifically evaluate cognitive functions following developmental exposure to concentrated ambient UFP exposure during the early postnatal period, considered equivalent to human third trimester of pregnancy (Clancy et al., 2007a,b; Rice and Barone, 2000) using measures of learning and memory (repeated learning and performance and novel object recognition, respectively), and impulsive-like behaviors (delay of reward and a differential reinforcement of low rates of response schedule). To determine the extent to which any potential learning, memory or impulsivity deficits in those paradigms might instead reflect altered motor function or altered motivation levels, measures of locomotor activity and progressive ratio performance were likewise included (Cory-Slechta, 1989; Cory-Slechta and Weiss, 2014). As we were able to expose mice to ambient CAPS concentrations in the current study that were only half of the concentration of our prior exposure concentrations (Allen et al., 2013), the current study also permitted assessment of behavioral deficits at levels of exposure lower than those in which CNS effects were previously observed and thus information on 'dose-response'.

2. Methods and materials

2.1. Animals and ultrafine particle exposure

Male and female C57Bl6/J mice were purchased from Jackson Laboratories (Bar Harbor, ME) and allowed to acclimate in the housing room for 1 week prior to breeding. Male and females were group housed prior to breeding. Estrous cycles of females were synchronized via pheromone-induced ovulation by placing females in cages with dirty bedding from males (Whitten, 1957). Monogamous pairs of mice were then bred for 3 days, males were removed and dams remained singly housed with litters until weaning. All animals utilized for this study were born in the same cohort between 8p.m.–6a.m. the following day to minimize ontogenetic variability and harmonize animal age. Mouse pups were exposed to concentrated (10–20 fold) ambient ultrafine particles (CAPS) using the Harvard University Concentrated Ambient Particle (HUCAPS) described below. As with human air pollution exposures, this includes day-to-day variability in particle mass concentrations as well as various adhering components. The gas-phase components of the ambient aerosol are present, but are not concentrated by the HUCAPS system.

Mice were removed from the dams and randomly assigned to exposures to CAPS or HEPA-filtered room air providing 99.99% effective Hepa filtration. To preclude litter specific effects, only a single pup, per time point, per sex, per litter, per squad was used in the study and pups were randomly assigned to respective treatment groups. Exposures lasted for 4 h/day between 1000 and 1400 h and were carried out at postnatal days (PND) 4–7 and 10–13 in compartmentalized whole-body inhalation exposure chambers using the HUCAPS System fitted with a size selective inlet which contains a high volume (5000 l/min) UFP concentrator to concentrate ambient particles generated from a nearby, highly trafficked road (Allen et al., 2014c). These exposure periods (PND4-7 and 10–13) were initially chosen based on their equivalence to human 3rd trimester (Clancy et al., 2007a,b; Rice and Barone, 2000) that represents a period of marked neuro- and gliogenesis (Bandeira et al., 2009) that were used in our prior studies demonstrating neuropathological and behavioral consequences of CAPS (Allen et al., 2014a,b,c), thus allowing direct comparisons to prior studies. These exposure levels are consistent with those reported for U.S. cities such as Raleigh, NC and Pittsburgh, PA (Kumar et al., 2014). Filtered air and CAPS- treated mice experienced similar experimental manipulations. Controls were exposed to HEPA-filtered air in an adjacent chamber. Both CAPS and control mouse chambers were maintained at 77–79 °F and 35–40% humidity.

Upon weaning at postnatal day 25, offspring were pair-housed by sex and treatment conditions (2–3/cage) under a 12 h light/dark cycle and temperature maintained at 72 °F. Behavioral testing commenced at approximately 35 days of age for initial locomotor testing (see Table 1). At approximately 60 days of age, all mice were reduced to 90% of their ad libitum weight to provide motivation for food-rewarded behavioral testing and maintained at those weights for the duration of the experiment. Because of the number of

Table 1
Sequential Order of Behavioral Testing, Paradigms Used and Age of testing for Each Squad.

Squad 1		Squad 2		Squad 3	
Behavioral Assay	Age (mos)	Behavioral Assay	Age (mos)	Behavioral Assay	Age (mos)
Locomotor Activity	1	Locomotor Activity	3.5	Locomotor Activity	7
Novel Object Recognition	1.5	Repeated Learning	4.5	Conditioned Place Preference	8
DRL Schedule	3.5	Progressive Ratio Schedule	7	Progressive Ratio Schedule	8
Progressive Ratio Schedule	6.5	Conditioned Place Preference	8	Delay of Reward Schedule	9
Elevated Plus Maze	7				

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