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Impact of prenatal polycyclic aromatic hydrocarbon exposure on behavior, cortical gene expression, and DNA methylation of the *Bdnf* gene ☆

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ABSTRACT

Prenatal exposure to polycyclic aromatic hydrocarbons (PAH) has been associated with sustained effects on the brain and behavior in offspring. However, the mechanisms have yet to be determined. We hypothesized that prenatal exposure to ambient PAH in mice would be associated with impaired neurocognition, increased anxiety, altered cortical expression of *Bdnf* and *Grin2b*, and greater DNA methylation of *Bdnf*. Our results indicated that during open-field testing, prenatal PAH–exposed offspring spent more time immobile and less time exploring. Females produced more fecal boli. Offspring prenatally exposed to PAH displayed modest reductions in overall exploration of objects. Further, prenatal PAH exposure was associated with lower cortical expression of *Grin2b* and *Bdnf* in males and greater *Bdnf IV* promoter methylation. Epigenetic differences within the *Bdnf* IV promoter correlated with *Bdnf* gene expression but not with the observed behavioral outcomes, suggesting that additional targets may account for these PAH-associated effects.

1. Introduction

Prenatal exposure to toxins can have a lasting impact on the brain and behavior with implications for development and health in subsequent generations (Skinner et al., 2008; Wolstenholme et al., 2012). Polycyclic aromatic hydrocarbons (PAH) are a class of pollutants produced during the incomplete combustion of organic materials. In US cities, traffic emissions are one of the most abundant sources of outdoor PAH. High levels of PAH exposure can originate from indoor sources (eg, space heating, cooking, smoking, burning incense or candles) as well as from outdoor traffic sources that penetrate indoors (Jung et al., 2010). In human cohort studies, our group at the Columbia Center for Children's Environmental Health (CCCEH) and others have shown that prenatal exposure to PAH was linked to reduced head circumference and birth weight (Dejmek et al., 2000), lower IQ (Perera et al., 2009), anxious and depressive symptoms, attention problems (Perera et al., 2012), reductions in white matter surface, and slower cognitive processing speed in later childhood (Peterson et al., 2015).

The molecular mechanisms of PAH-induced disruption to neurodevelopment have yet to be determined. In laboratory rodents, prenatal exposure to low doses of the PAH benzo[a]pyrene impaired learning and memory, in part by reducing neural plasticity by disrupting glutamate signaling (Brown et al., 2007) and inducing toxic effects to glial cells (Dutta et al., 2010; Wormley et al., 2004). Environmentally induced alterations in DNA methylation within the brain that may suppress gene expression have been demonstrated following prenatal exposure to stress (Mueller and Bale, 2008), endocrine-disrupting chemicals (Kundakovic et al., 2013), and variation in the nutritional environment (Cho et al., 2013) and may serve as a molecular mechanism that mediates the sustained effects of prenatal and postnatal PAH environmental exposures. For example, in vitro studies demonstrated transcriptional changes of interspersed element-1 (LINE-1), a mammalian retrotransposon, in response to benzo[a]pyrene exposure; these were attributed to reductions in the expression of DNA methyltransferase-1 (*Dnmt1*) and *Dnmt1*

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recruitment to the LINE-1 promoter (Teneng et al., 2011). In zebrafish, exposure to benzo[a]pyrene was shown to induce both genomewide transcriptional changes in embryos (Fang et al., 2015) and gene-specific and genomewide reductions in DNA methylation (Fang et al., 2013). In human cohorts, global reductions in DNA methylation cord blood were associated with prenatal exposure to elevated PAH levels (Herbstman et al., 2012). Overall, these studies provide converging evidence implicating epigenetic changes in mediating the effects of PAH.

In the current study, we conducted analyses of the impact of prenatal exposure to PAH on behavior, brain gene expression, and DNA methylation in mice exposed to an aerosolized mixture that simulates the prenatal PAH exposure of a CCCEH cohort of children from Northern New York City (Chu et al., 2013; Yan et al., 2014). Within this study, we assessed behavioral outcomes related to cognition, activity levels, and anxiety-like phenotypes and focused on the expression of 2 candidate genes implicated in neural plasticity: brain-derived neurotrophic factor (*Bdnf*) and the ionotropic glutamate receptor *N*-methyl D-aspartate 2B (*Grin2b* or *Nmdar2b*). Previous studies have demonstrated altered expression and DNA methylation of *Bdnf* exon IV in response to in utero exposure to endocrine-disrupting chemicals in mice and humans (Kundakovic et al., 2015) and postnatal exposure to stressful rearing conditions in rats (Roth et al., 2009). In humans, prenatal exposure to related combustion products, namely, fine particulate matter, was associated with lower *Bdnf* expression in the placenta (Saenen et al., 2015). Closure of a highly polluting, coal-fired power plant was associated with higher mature BDNF protein levels in cord blood and improved neurocognitive development measured at 2 years of age (Tang et al., 2014). Similarly, studies in rodents indicated altered *Grin2b* expression and promoter DNA methylation in response to prenatal adversity (Kundakovic et al., 2015; Kleiber et al., 2013), and *Grin2b* genetic variations predicted risky decision making and attention problems in human cohorts (Riva et al., 2015; Ness et al., 2011).

We hypothesized that prenatal exposure to PAH would be associated with impaired neurocognitive function and increased anxiety-like behaviors. Second, we hypothesized that prenatal exposure to PAH would be associated with decreased expression of *Bdnf* and *Grin2b* in the cerebral cortex and increased DNA methylation of *Bdnf* IV. Finally, based on previous studies indicating sex differences in neurodevelopmental and epigenetic outcomes associated with prenatal exposure to stress and endocrine disrupting chemicals (Mueller and Bale, 2008; Kundakovic et al., 2013), we predicted that both behavioral and gene expression/epigenetic outcomes associated with prenatal exposure to PAH would vary by offspring sex.

2. Materials and methods

2.1. Animals

Nine-week-old BALB/cByj female mice weighing 20-22 g were obtained from Charles River and housed 4-5/cage in temperature-regulated (20°C), ventilated cabinets with a 12-hour light/12-hour dark cycle (09:00 to 21:00). Animals were provided ad libitum access to a standard diet and water and were housing acclimated in this controlled environment for at least 1 week before any experiments. After 1 week, mice were mated, and the protocol summarized in Fig. 1 was initiated. Animal experiments were carried out in strict accordance with the principles and procedures of the *Guide for the Care and Use of Laboratory Animals*. The protocol was approved by the Institutional Animal Care and Use Committee, Columbia University Medical Center.

2.2. PAH exposure

The PAH mixture was produced by the Lovelace Respiratory Research Institute to replicate the proportions of individual PAH that was measured among a cohort of more than 700 pregnant women using personal air sampling devices (Chu et al., 2013; Yan et al., 2014; Miller et al., 2004). Briefly, the negative control aerosol solution consisted of 99.97% purified water, 0.02% Tween 80, and 0.01% antifoam (Sigma-Aldrich, St Louis, MO). The mixed PAH solution was added to yield the final concentration of 7.29 ng/m^3. Solutions were delivered via nebulizers (Unomedical Inc, McAllen, TX) connected to filtered compressed air in a chamber set to achieve a flow of 12.5 to 13.0 L/min (Chu et al., 2013; Yan et al., 2014). Four dams were exposed in 1 cage at a time with full access to food and water for 5 hours a day, 5 days a week, beginning on gestational day 0.5-2.5 until birth of litter (gestational day 19-21). Upon completion of the daily exposure session, mice were returned to their usual housing conditions. Previously, we determined chamber pyrene levels of 23.24 ± 3.05 ng/m^3 (range, 7.38-40 ng/m^3) from 3 weekly filters extracted together (Chu et al., 2013), suggesting that levels ambient in the chamber may be higher than levels ambient in the NYC urban environment. Prenatally exposed offspring were weaned at postnatal day 21 and housed in same-sex, same-condition groups of 4 mice per cage until testing.

2.3. Behavioral phenotyping

At postnatal day 60, male and female offspring (sample sizes for behavioral analyses consisted of n = 18 male/n = 18 female control offspring and n = 14 male/n = 16 female PAH-exposed offspring) were assessed on a battery of behavioral tests that included indices of activity, exploration, anxiety-like responses, and recognition memory (Fig. 1). Tests were conducted sequentially with 3-4 days between tests in the following order: open field, elevated plus, light-dark box, and novel object recognition.

2.3.1. Open-field testing

Behavior during open-field assessment is a standard measure of activity and anxiety-like responses in rodents (Belzung and Griebel, 2001). The open-field apparatus used was a 24 × 24 × 16-in Plexiglas box. On the day of testing, the mouse was placed directly into one corner of the open-field. After a 10-minute session, the mouse was returned to its home cage. All testing was conducted under red lighting conditions. Behaviors were video recorded. Behaviors scored using AnyMaze software (version 4.82) included (1) total distance traveled (meters), (2) time spent immobile (seconds), and (3) time spent in the center area (the inner 12 × 12-in region of the field) (seconds). Counts of fecal boli produced during testing also were assessed.

2.3.2. Elevated plus maze

The elevated plus maze is a standard measure of anxiety-like behavior and exploration in rodents (Walf and Frye, 2007). The

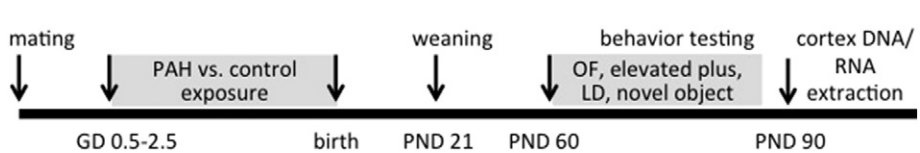

Fig. 1. Study design indicating timing of treatment and assessments of offspring. Abbreviations: OF = open field; LD = light-dark box; GD = gestational day; PND = postnatal day.