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Full Length Article

Effect of neonatal hyperoxia followed by concentrated ambient ultrafine particle exposure on cumulative learning in C57Bl/6J mice

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ABSTRACT

Hyperoxia during treatment for prematurity may enhance susceptibility to other risk factors for adverse brain development, such as air pollution exposure, as both of these risk factors have been linked to a variety of adverse neurodevelopmental outcomes. This study investigated the combined effects of neonatal hyperoxia followed by inhalation of concentrated ambient ultrafine particles (CAPS, < 100 nm in aerodynamic diameter) on learning. C57BL/6 J mice were birthed into 60% oxygen until postnatal day (PND) 4 and subsequently exposed to filtered air or to CAPS using the Harvard University Concentrated Ambient Particle System (HUCAPS) from PND 4–7 and 10-13. Behavior was assessed on a fixed interval (FI) schedule of reinforcement in which reward is available only after a fixed interval of time elapses, as well as expected reductions in behavior during an extinction procedure when reward was withheld. Both produce highly comparable behavioral performance across species. Performance measures included rate of responding, response accuracy, and temporal control (quarter life). Exposure to hyperoxia or CAPS resulted in lower mean quarter life values, an effect that was further enhanced in males by combined exposure, findings consistent with delayed learning of the FI schedule. Females also initially exhibited greater reductions in quarter life values following the combined exposure to hyperoxia and CAPS and delayed reductions in response rates during extinction. Combined hyperoxia and CAPS produced greater learning deficits than either risk factor alone, consistent with enhanced neurodevelopmental toxicity, findings that could reflect a convergence of both insults on common neurobiological systems. The basis for sex differences in outcome warrants further research. This study highlights the potential for heightened risk of adverse neurodevelopment outcomes in individuals born preterm in regions with higher levels of ultrafine particle (UFP) air pollution, in accord with the multiplicity of risk factors extant in the human environment.

1. Introduction

Risk factors associated with prematurity include hyperoxia (Deulofeut et al., 2006), ischemia (Vohr et al., 2000), infection (Stoll et al., 2004), and thermoregulatory impairments (Simbruner et al., 2010), all of which can lead to cognitive dysfunction. Advances in medical care over the past two decades have increased survival and prognosis for very preterm infants (< 32 weeks) (Stoll et al., 2015). Successful survival of these infants, however, raises additional concerns surrounding their unique challenges and susceptibilities. One early environmental insult of concern is exposure to air pollution, which itself is associated with risk of prematurity (Laurent et al., 2016), and as such, pre-term infants may be returning to homes in higher air pollution environments. Thus, the CNS injuries that can occur in children born prematurely may be followed by exposure to other risk factors for adverse neurodevelopment, such as air pollution.

Air pollution is considered a global health risk, and represents a heightened threat to vulnerable populations (Cohen et al., 2017). Air pollution is a heterogeneous mixture of particles, gases, and organic compounds, many of which have the potential to alter CNS development. Of particular concern with regard to the CNS is the ambient ultrafine particle (UFPs; < 100 nm in diameter) component of this mixture. UFPs are capable of directly translocating into the CNS via uptake into nerve terminals in the olfactory mucosa and subsequently depositing within the brain parenchyma where they can produce neuroinflammation (Elder et al., 2006; Oberdorster et al., 2004). UFPs can also exert effects via indirect mechanisms, including long-term retention in the lung, which can trigger chronic inflammation (Park et al., 2015; Sun

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et al., 2012) and potentially lead to systemic inflammation. Our laboratory has shown that early neonatal exposure to concentrated ultrafine ambient particles (CAPS) can produce neuropathological changes indicative of a neuroinflammatory response, including ventriculomegaly, microglial activation, pro-inflammatory cytokine changes and delayed-white matter development (Allen et al., 2014a, 2015), highlighting the susceptibility of the developing CNS to the consequences of air pollution.

Preterm infants undergo a unique, untimely transition from an appropriate low oxygen, hypoxic *in utero* environment to a high oxygen clinical environment to ensure their survival (Gao and Raj, 2010; Sola, 2015). During this transition, it is common for oxygen blood saturation levels to fluctuate above clinical targets in preterm infants during long-term oxygen supplementation, with infants in some facilities spending up to 50% of their time in hyperoxic conditions (Deulofeut et al., 2006; Sink et al., 2011). Neonatal hyperoxia in preterm infants is well-established as a contributor to bronchopulmonary dysplasia (BPD), a chronic respiratory disease characterized by simplified alveolar structure and restrictive airways (Domm et al., 2015). More evidence is emerging that hyperoxia also contributes to CNS deficits, as duration of oxygen support for preterm infants has been linked to decreased cortical growth (Bouyssi-Kobar et al., 2016) and increased cerebral oxygenation following birth has also been linked to poor cognitive outcomes measured using the Bayley Scales (Verhagen et al., 2015). Neonatal hyperoxia exposure in rodents decreases cortical growth (Sirinyan et al., 2006), disrupts white matter growth (Ritter et al., 2013), and leads to glial activation (Schmitz et al., 2011; Vottier et al., 2011).

The adverse consequences of a hyperoxic environment on the developing CNS could conceivably be compounded by subsequent exposure to air pollution, given its targeting of the developing CNS and the fact that like hyperoxia, it too can impair cognitive functions, as human brain development remains significant nearly into adulthood (Arnold, 2009; Vertes and Bullmore, 2015). Prematurity has been linked to a variety of adverse neurodevelopmental clinical outcomes including autism spectrum disorder (ASD) (Jarjour, 2015), attention deficit hyperactivity disorder (ADHD) (Bhutta et al., 2002), schizophrenia (Dalman et al., 1999) and cognitive dysfunction (Soria-Pastor et al., 2008), neurodevelopmental disorders that have also been linked to early air pollution exposures. Exposures to traffic-related (Becerra et al., 2013; Volk et al., 2011) and $PM_{2.5}$ (Volk et al., 2013) air pollution exposure during the perinatal period have been associated with an increased risk for ASD in Los Angeles. Elevated ambient PM_{10} exposure during childhood was associated with increased prevalence of ADHD in India (Siddique et al., 2011). Increased black carbon exposure of children in Boston, Massachusetts was associated with decreased scores in verbal and nonverbal memory assessment (Suglia et al., 2008). Increased $PM_{2.5}$ exposure was associated with reductions in cognitive growth and working memory in a prospective cohort of schoolchildren in Barcelona, Spain (Basagana et al., 2016). The similarity of the adverse neurodevelopmental outcomes of hyperoxia and UFPs and their potential for sequential occurrence raises the possibility that these exposures could produce cumulative neurodevelopmental risk, and underscores the need to consider their combined effects. The imposition of UFP exposure post hyperoxia exposure at birth therefore embodies a more real-world environmental exposure scenario and thus is of greater translational relevance.

To understand the potential for enhanced neurodevelopmental risk, this study used a model of neonatal hyperoxia followed by CAPS exposure in mice. As CNS development in rodents at birth is equivalent to the early third-trimester in humans (Semple et al., 2013), this model effectively resembles the CNS developmental stage of preterm infants exposed to hyperoxia. Learning was assessed by examining acquisition of prototypical behavior on a fixed-interval (FI) schedule of food reward which provides access to reward contingent upon the first occurrence of a designated response that occurs after a specified fixed interval of time has elapsed; responses prior to the completion of the interval have no consequence and cannot accelerate time to reward availability. The characteristic behavioral pattern controlled by this schedule occurs across a wide range of species (Kelleher and Morse, 1968) and relies on temporal control, i.e, it ultimately consists of little or no responding early in the interval followed by maximal rates of responding as the end of the interval approaches to minimize delay of reward, thus necessitating a temporal discrimination by the organism. Temporal control increases with age (Brannon et al., 2007), and is correlated with IQ (Chelonis et al., 2004). Measuring temporal behavior on the FI schedule using a quarter life measure (time during the specified interval at which 25% of the responses occurred), as well as reductions in response rate when reward was subsequently withheld during extinction, this study sought to assess whether combined neonatal hyperoxia and CAPS exposure would result in enhanced learning deficits.

2. Materials and methods

2.1. Animals and exposure paradigm

Adult male and female C57BL/6 J mice from Jackson Laboratories (Bar Harbor, ME) were bred using a scheme designed to ensure timed births as previously described (Allen et al., 2014a). Newborn C57BL6 mice were birthed and maintained at 60% oxygen conditions until neonatal day 4, then maintained under normal animal room oxygen levels (21%). For this purpose, pure oxygen was humidified with sterile distilled water to 40–70%, filtered, and passaged into the chambers before venting out of the building. Mice birthed into room air served as controls. Because adult mice are sensitive to hyperoxia, dams were rotated every 24 h between litters exposed to room air or hyperoxia. This exposure paradigm has been described and further detailed previously (Yee et al., 2009). A total of 46 litters were exposed to room air or hyperoxia and then divided so that pups from each litter were randomly assigned to subsequent exposure to CAPS or filtered air (Air) in a counterbalanced order that precluded litter-specific effects.

Following hyperoxia exposure, mice were removed from dams and exposed to CAPS, as described previously (Allen et al., 2013, 2014a). Neonatal mice were exposed to filtered air or ambient UFP (< 100 nm in diameter) concentrated 10–20 fold using the Harvard Ultrafine Concentrated Ambient Particle System (HUCAPS). Exposures lasted for 4 h per day beginning at 9:00 a.m. on PND (postnatal day) 4–7 and 10–13. During these exposures, pups were housed in small mesh chambers with four pups per chamber. This high-volume ambient sampling system utilizes condensational growth of the particulate phase in conjunction with virtual impaction to provide aerosols that are enriched for sizes smaller than 200 nm in diameter, with median sizes being typically in the 70–90 nm range with concentrations of approximately $0.2–2 \times 10^5/cm^3$. The gas-phase components of the ambient aerosol are not concentrated by the HUCAPS system. Particle counts were obtained using a condensation particle counter (model 3022 A;TSI, Shoreview, MN), and mass concentration calculated using idealized particle density ($1.5\ g/cm^3$). PTFE filters (47 mm, 0.2 μm pore size, Pall, Port Washington, New York) were collected daily from the filtered air and HUCAPS exposure chambers for analysis of elemental composition using x-ray fluorescence (XRF). The hyperoxia and CAPS exposures generated 4 treatment groups per sex: neonatal hyperoxia with and without CAPS (designated H Air and H CAPS, respectively) and neonatal room air with and without CAPS (designated A Air and A CAPS, respectively). No more than 1–2 pups per litter/per sex were assigned to any given treatment group to preclude litter effects, and each treatment group had $n = 10$ except for female A CAPS, where $n = 9$. All experimental activities were approved by the University of Rochester Institutional Animal Care and Use Committee.