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Exposures to fine particulate matter ($PM_{2.5}$) and ozone above USA standards are associated with auditory brainstem dysmorphology and abnormal auditory brainstem evoked potentials in healthy young dogs

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ABSTRACT

Background: Delayed central conduction times in the auditory brainstem have been observed in Mexico City (MC) healthy children exposed to fine particulate matter ($PM_{2.5}$) and ozone (O_3) above the current United States Environmental Protection Agency (US-EPA) standards. MC children have α synuclein brainstem accumulation and medial superior olivary complex (MSO) dysmorphology. The present study used a dog model to investigate the potential effects of air pollution on the function and morphology of the auditory brainstem.

Methodology: Twenty-four dogs living in clean air v MC, average age 37.1 ± 26.3 months, underwent brainstem auditory evoked potential (BAEP) measurements. Eight dogs (4 MC, 4 Controls) were analysed for auditory brainstem morphology and histopathology.

Results: MC dogs showed ventral cochlear nuclei hypotrophy and MSO dysmorphology with a significant decrease in cell body size, decreased neuronal packing density with regions in the nucleus devoid of neurons and marked gliosis. MC dogs showed significant delayed BAEP absolute wave I, III and V latencies compared to controls.

Conclusions: MC dogs show auditory nuclei dysmorphology and BAEPs consistent with an alteration of the generator sites of the auditory brainstem response waveform. This study puts forward the usefulness of BAEPs to study auditory brainstem neurodegenerative changes associated with air pollution in dogs. Recognition of the role of non-invasive BAEPs in urban dogs is warranted to elucidate novel neurodegenerative pathways link to air pollution and a promising early diagnostic strategy for Alzheimer's Disease.

1. Background

Air pollution is responsible for over 5.5 million premature deaths and 141.5 million years of disability-adjusted life years in 188 countries (Forouzanfar et al., 2015). Environmental pollutants, toxic industrial chemicals, pesticides, heavy metals, and hazardous wastes have detrimental, sometimes irreversible impact upon the developing central nervous system in children (Calderón-Garcidueñas et al., 2012a, 2016a; Calderon-Garciduenas and de la Monte, 2017; Miller et al., 2016). Traffic-related pollution has increasingly been identified as an

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important contributor to adverse health effects of air pollution and its impact extends from pregnancy to adult life (Kolosnjaj-Tabi et al., 2015; Stieb et al., 2016; Basagaña et al., 2016; Wu et al., 2016; Porta et al., 2016; Chen et al., 2017; Calderón-Garcidueñas et al., 2015a, 2015b).

Air quality in Mexico City (MC) has been recognized as among the worst in the world (Bravo-Alvarez and Torres-Jardón, 2002; Parrish et al., 2011). Despite a number of actions and restrictions imposed to curtail the air pollution during the past two decades, levels of fine particulate matter ($PM_{2.5}$) and O_3 (ozone) have stayed significantly above the US EPA standards (US EPA, 2014), leading to continuously high exposures of today's children and teenagers, starting *in utero* (Bravo-Alvarez and Torres-Jardón, 2002; De Vizcaya-Ruiz et al., 2006; Querol et al., 2008; Davis, 2017).

Brainstem Auditory Evoked Potentials (BAEPs) represent a reliable means of evaluating the integrity of the auditory nerve and brainstem (Strain et al., 1991; Wilson and Mills, 2005; Wilson et al., 2006, 2011) and providing information of the neurologic and audiologic status of healthy and high risk populations, including residents in highly polluted megacities (Scaioli et al., 2009; Calderón-Garcidueñas et al., 2011). For example we have reported that BAEPs in clinically healthy children (8.05 ± 1.4 year old) in Southwest Mexico City (SWMC) had significant delays in wave III ($t(50) = 17.038$; $p < .0001$) and wave V ($t(50) = 19.730$; $p < .0001$), and also significantly longer inter-wave intervals for waves I-III, III-V, and I-V (all $t(50) > 7.501$; $p < .0001$) versus clean air controls (Calderón-Garcidueñas et al., 2011). These findings are consistent with delayed central conduction time of brainstem neural transmission. These SWMC children also exhibited significant evidence of neuroinflammation, accumulation of α synuclein in auditory and vestibular nuclei, significant dysmorphology in medial superior olive (MSO) neurons, and alpha-synuclein neuronal aggregation (the early neuropathological hallmark of sporadic Parkinson's disease) (Calderón-Garcidueñas et al., 2011). The observed BAEPs abnormalities in SWMC children were interpreted as a sign of auditory neuronal injury associated with both neuroinflammation and neurodegeneration. This was thought to place these children at high risk for auditory and vestibular impairment, particularly since none of these abnormalities were present in matched control children (Calderón-Garcidueñas et al., 2011).

Indications that air pollution could affect brain function in children warrant further investigation using models that allow for greater integration of BAEP, morphological and histopathological data. One such model is the dog, an animal on which there is an established history of BAEP research (Sims, 1988; Fischer and Obermaier, 1994; Wilson and Mills, 2005; Wilson et al., 2006, 2011; Schmutz, 2014). The use of a dog model would allow researchers to study dog cohorts with complete medical and air pollution exposure data. This would allow for the study of associations amongst BAEP results, auditory brainstem morphology and pathology, $PM_{2.5}$ and O_3 exposures. Our previous work revealed significant dysmorphology in the human medial superior olive (MSO), an essential auditory brainstem center (Calderón-Garcidueñas et al., 2011). Further, this nucleus appears to be susceptible to neurodevelopmental disorders (Kulesza and Mangunay, 2008; Kulesza et al., 2011; Lukose et al., 2015). Because of the abnormalities in auditory brainstem response observed in humans, we therefore chose to examine the dogs' MSO morphology.

In the present study, we aimed to determine if: 1. Dogs living in polluted air showed signs of abnormal auditory brainstem morphology and/or histopathology compared with dogs in clean air cities. 2. Dogs living in polluted air showed abnormal BAEP waveforms compared to dogs living in clean air.

Abnormal BAEP measurements are linked with brainstem nuclei dysmorphology in dogs. Similar findings have been reported in children and young adult people who have been exposed to high levels of particulate matter and ozone: Could BAEPs abnormalities in young urbanites be a proxy for an evolving neurodegenerative process towards Alzheimer's disease (AD)? We argue the answer is yes.

2. Methods

2.1. Study areas

Two study regions were used in this study: Mexico City (MC) and Tlaxcala, a control city. MC is the largest urban center in North America and is a good example of extreme urban growth (Bravo-Alvarez and Torres-Jardón, 2002; Parrish et al., 2011; Calderón-Segura et al., 2004; Dzepina et al., 2007; Valle-Hernández et al., 2010; Mugica-Álvarez et al., 2012; US EPA, 2014; Davis, 2017).

Its metropolitan area of over 2000 square kilometers lies in an elevated basin 2240 m above sea level surrounded on three sides by mountain ridges, a broad opening to the north, and a narrower gap to the south-southwest. Dogs (and humans) living in Southern Mexico City have been exposed to significant concentrations of O_3 and aerosols resulting from typical diurnal wind transportation of air masses rich in these secondary pollutants towards smog receptor sites in southern MCMA. In addition, they have high exposures to secondary tracers (NO_3^-) and particles with lipopolysaccharides (PM-LPS); volatile organic compounds (VOCs); $PM_{2.5}$ and its constituent organic and elemental carbon including polycyclic aromatic hydrocarbons; secondary inorganic aerosols (SO_4^{2-}, NO_3^-, NH_4^+); and metals (Zn, Cu, Pb, Ti, Mn, Cr, V), (Figs. 1–3) (Dzepina et al., 2007; Querol et al., 2008; Valle-Hernández et al., 2010; Vega et al., 2010). Finally, they also have high exposures to total PAHs including benzo [a] pyrene (Marr et al., 2006). These semi-volatile compounds are emitted mainly by uncontrolled sources such as diesel motor vehicle, biomass and garbage burning (Fig. 4). The control city has relatively few contributing emission sources from industry and cars and good ventilation conditions due to regional winds and the criteria pollutants are all below USEPA standards (US EPA, 2014; Calderón-Garcidueñas et al., 2001, 2002, 2003).

3. Experimental animals

The study protocol was approved by the Institutional Animal Care and Use Committee at the Instituto Nacional de Pediatría (INP). Procedures used were in accordance with the guidelines of the INP on the Use and Care of the Animals, the regulations of the NOM-062-ZOO-1999 Official Mexican Standard and the Guide for the Care and Use of Experimental Animals and the standard in the Guide for the Care and Use of Laboratory Animals (NIH publication No. 86-23).

The INP provided full veterinary daily care of the dogs included in

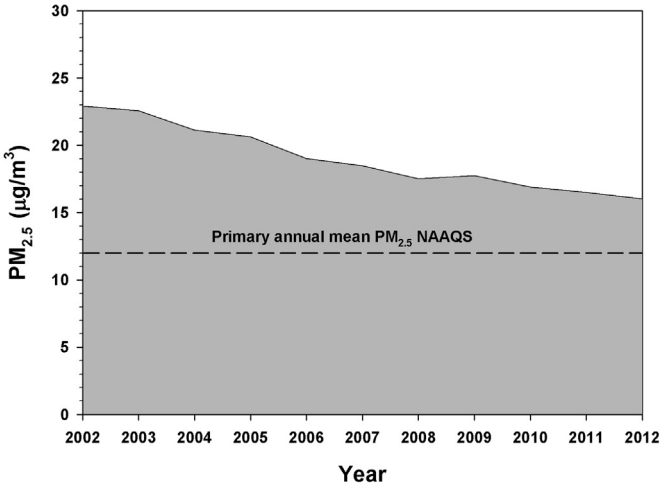

Fig. 1. Data from three year average fine particulate matter $PM_{2.5}$ annual mean concentrations registered at the southwest Mexico City from 2002 to 2012. The dashed line represents the annual $PM_{2.5}$ US-EPA National Air Quality Standard. Since $PM_{2.5}$ measurements began in 2004, data previous to this year were obtained from the slope of the correlation PM_{10} v $PM_{2.5}$. Data concentrations were obtained from the Air Quality Monitoring Network of the Government of Mexico City.