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Estimating the causal effect of prenatal lead exposure on prepulse inhibition deficits in children and adolescents

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ABSTRACT

During pregnancy, maternal lead from earlier exposures mobilizes and crosses placental barriers, placing the developing fetus at risk for lead exposure and neurodevelopmental deficits. Some neuronal circuits known to be affected in neurodevelopment disorders can be probed with simple physiological behavioral paradigms. One such neural biomarker is Pre-Pulse Inhibition (PPI), an indicator of adequate sensorimotor gating processing. In clinical studies, deficits in PPI have been associated with neurodevelopmental disorders in human subjects. To our knowledge, no studies have examined the use of PPI as a biomarker of toxicant effects on the brain in epidemiological studies. We aimed to estimate the causal effect of prenatal lead exposure, assessed by maternal cortical bone lead concentrations, on PPI in 279 children from Mexico City. *in vivo* maternal cortical bone lead measurements were taken at four weeks postpartum at the mid-tibia shaft using a K-Shell X-ray fluorescence instrument. PPI recording occurred in an isolated clinical setting and eye blink responses were measured using electromyography. We assessed if the conditions for causal inference held in our study and used the results of our assessment to estimate the causal effect of prenatal lead exposure on PPI using an ordinary least squares regression model, a marginal structural model, and the parametric g-formula. Results were consistent across the three modeling approaches. For the parametric g-formula, a one standard deviation (10.0 μg/g) increase in prenatal lead significantly reduced PPI by approximately 19.0 % (95 % CI: 5.4 %, 34.3 %). This decrease is similar in magnitude to clinical studies on schizophrenia, which have observed PPI impairments in patients with schizophrenia as compared to controls. Our results are consistent with findings from other studies establishing an association between lead exposure and neurodevelopmental disorders in children and suggest that PPI may be useful as an objective biomarker of toxicant effects on the brain.

1. Introduction

Lead is a highly toxic heavy metal known to interfere with brain development in children (Hong et al., 2014; Lidsky and Schneider, 2003). In pregnant women, lead from earlier exposures can be released into the bloodstream, which can subsequently cross the placenta and place the developing fetus at risk for lead-related neurobehavioral deficits (Bornschein et al., 1977; Korpela et al., 1986; Lidsky and Schneider, 2003; Manton et al., 2003). After exposure, lead crosses the blood brain barrier and deposits in the developing fetus's brain, primarily because of its ability to substitute for calcium. There, it can alter the behavior of endothelial cells in the immature brain and disrupt the blood brain barrier, as well as directly interfere with neuronal signaling (Sanders et al., 2009). Research findings have suggested that prenatal lead exposure is associated with reduced cognition (Howard Hu et al., 2006; Schnaas et al., 2006; Wasserman et al., 2000), attention

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(Wasserman et al., 2000), and altered auditory and retinal function (Dietrich et al., 1992; Rothenberg et al., 2000; Wasserman et al., 2000) in children.

Specific neuronal circuits known to be affected in several neurodevelopment disorders can be probed with simple physiological behavioral paradigms, potentially serving as a marker of neurobehavioral impairments associated with exposure to environmental metals (Rowland et al., 2002). One such measure is pre-pulse inhibition (PPI), a sensorimotor gating process that can modify the acoustic startle reflex (ASR) under different behavioral conditions (e.g. during attention tasks; Hawk W. Larry, Pelham E. William, & Yartz R. Andrew, 2002). The ASR is a cross-species, whole-body reflex in response to a loud and unexpected sound that has been well characterized as an element of a defensive response to stressful stimuli (Le Duc et al., 2016). The ASR is attenuated when a quieter, non-startling sound (a "prepulse") is presented 30–500 milliseconds (ms) before the startle probe (D. L. Braff et al., 2001). This attenuation, termed PPI, is an indication of early automatic attention regulation of environmental stimuli (Le Duc et al., 2016). Since PPI indicates adequate organization of human cognitive processes, it has been proposed that PPI deficits can be used as a neurobiological marker for pathologies indicative of inadequate motor or sensory gating such as observed in schizophrenia and other neurodevelopmental disorders (D. Braff et al., 1978; De la Casa et al., 2016). In children, PPI deficits are associated with neurodevelopmental disorders such as early psychosis, Tourette's syndrome, primary nocturnal enuresis (Takahashi et al., 2011), and DiGeorge syndrome (Sobin et al., 2005).

Although PPI's relevance for neurodevelopmental disorders has been established, its relationship with environmental neurotoxicants such as lead has not been epidemiologically explored. Yet, by probing underlying neurobiology, PPI offers the potential to identify neurological deficits associated with neurotoxicant exposures. Moreover, as an indicator of the underlying biological processes possibly implicated in neurodevelopmental disorders, PPI may be able to identify deficits earlier using more objective measures than behavioral ratings and can be more cost-effective than current brain imaging techniques for assessing the neurobiology of these disorders in larger epidemiological samples.

To our knowledge, no studies have examined the effect of prenatal lead exposure on PPI in children. The objective of this study was to estimate the causal effect of prenatal lead exposure on PPI. To do so, we emulated a randomized control trial of prenatal lead exposure in a cohort of children 8–17 years of age from Mexico City. We applied traditional least squares regression models, marginal structural models, and the parametric g-formula to estimate the effect of prenatal lead exposure on PPI in children and compared the consistency of our effect estimates across the three modeling approaches.

2. Methods

2.1. Study population

The Early life Exposure in Mexico to Environmental Toxicants (ELEMENT) cohort consists of three sequential birth cohorts of mother-infant pairs from Mexico City maternal hospitals that have been followed for over two decades to understand health effects associated with environmental exposures to metals and chemicals (Téllez-Rojo et al., 2006). Pertinent details of ELEMENT, such as inclusion and exclusion criteria, collection methods, and demographics have been reported in detail elsewhere (Afeiche et al., 2011; Téllez-Rojo et al., 2006). In brief, 2098 pregnant women were recruited from prenatal clinics in Mexico City, a catchment population of low-to-middle income individuals formerly employed in the private sector. Cohort I was recruited from 1994 to 1997, Cohort II from 1997 to 2000, and Cohort III from 2001 to 2005. The ELEMENT cohort consists of the 2098 mothers and 1710 children. Between 2008 and 2011, 827 children participated in a follow-up study on heavy metals and attention behaviors. Of these 827 participants, 415 children 8–17 years old subsequently participated in the current PPI sub-study approximately 18 months later.

The research protocol for this study was carried out in accordance with the Declaration of Helsinki and approved by the ethics and research committees of the partnering institutions, including the National Institute of Public Health of Mexico, Harvard T.H. Chan School of Public Health, Brigham and Women's Hospital, the University of Michigan School Of Public Health, the University of Washington, and other participating hospitals. Informed consent was obtained from each participant.

2.2. Exposure assessment

Prenatal blood lead measurements were only available for mothers in Cohort III and a fraction of mothers in Cohort II. As such, we assessed prenatal lead exposure by using cortical bone lead (μg/g of bone mineral) measured at 4 weeks postpartum (± 5 days) at the mid-tibial cortical bone shaft in mothers. Although trabecular bone lead was measured at the same time for mothers, our analyses focus on cortical bone lead as it has been highlighted as the more reliable measure of prenatal lead exposure, due to the high turnover expected with trabecular bone lead during and after pregnancy (Afeiche et al., 2011; Howard Hu et al., 2006). We use the term "prenatal lead" to refer to cortical bone lead measurements in subsequent sections of this article.

Bone lead was measured non-invasively using a spot-source ^{109}Cd-based K-shell X-ray fluorescence (K-XRF) instrument maintained at Harvard University and installed in a research facility in the American British Cowdray Medical Center in Mexico City. The physical principles, technical specifications, and validation of this and other similar K-XRF instruments have been described previously (Aro et al., 1994; Burger et al., 1990; Hu et al., 1998).

2.3. Covariate information

ELEMENT has detailed information on maternal, child, and familial characteristics dating back to pregnancy. In addition, we collected updated questionnaire-based information on maternal marital status, parental education levels, and family socioeconomic status at recruitment for the child follow-up study. The socioeconomic questionnaire asked about the availability of certain items and assets in the home (number of light bulbs in the home, rooms in the house, bathrooms, cars, personal computer, water heater, electrical appliances [video/DVD player, washing machine, vacuum cleaner, toaster, microwave], and the type of house floor). Point values were assigned to each item, and the socioeconomic status level was calculated based on the sum of the points across all items (Huang et al., 2015). This approach was developed by the Asociación Mexicana de Agencias de Investigación de Mercados y Opinión Pública (Carrasco, 2002).

2.4. PPI experimental design

The specifics of this startle procedure are based on those previously used in assessment of attentional modulation of PPI in children and adolescents (Hawk et al., 2003; Hawk W. Larry et al., 2002). Before the trials, participants were placed in an isolated clinical setting and surface electrodes were placed over each child's orbicularis oculi muscle to measure eye blink response. Acoustic stimuli were delivered through headphones, where background white noise of 70 dB was played continuously. An initial series of two different tones were presented over the headphones and each child was instructed to discriminate between high (1200 Hz) and low (400 Hz) pitches, and short (5 s) and long (8 s) tones in random order. The discrimination exercise was repeated until each participant responded correctly to six consecutive stimuli to ensure that each child could discriminate the differences.

After the discrimination exercise, the experiment commenced and

ran continuously until completion as a continuous recording. A subsequent session immediately following the discrimination exercise consisted of the presentation of a series of tones each followed by an inter-trial interval (ITI) during which no tones were presented, with the two-tone pitches (400 Hz and 1200 Hz) and lengths (5 s and 8 s) presented in pseudo-randomized order to counterbalance tone order. The child was instructed to attend to one of the pre-pulse tones (target as either high or low pitch selected randomly) and to ignore the other (non-target). Participants were instructed to press a button at the end of all the longer (8 s) presentations of the attended (target) pre-pulse tone. Startle probes at 102 dB were presented during some of the pre-pulse tones and during some of the ITIs. The length of the ITIs was either 15, 22, or 29 s long and startle probes were presented 7, 11, 14 s into those intervals, respectively. Each child was exposed to 12 startle probes that appeared in pseudorandom order with respect to target, non-target, or ITI. In total, there were six trials with target tones, six trials with non-target tones, and 12 ITIs. For the target and non-target tones, startle probes were presented either 120 or 240 ms after the start of the target or non-target tone (stimulus onset asynchrony or SOA). Over the whole session a total of four startle probes were presented during each of the conditions (target tone, non-target tone, or ITI).

2.5. Signal recording and electromyography data processing

The specifics of signal recording electromyography (EMG) data processing for this study were consistent with guidelines outlined by Blumenthal et al. for human startle eye blink electromyography studies (Blumenthal et al., 2005). Eye blink responses to startle stimuli were recorded using a bipolar configuration of 3 mm Ag-AgCl surface electrodes filled with Microlyte Gel (Coulbourn, Allentown PA) secured by adhesive collars positioned over the orbicularis oculi muscle beneath the lower eyelid, one below and in line with the pupil in a forward gaze and the other 1 − 2 cm lateral to the first. The startle delivery and response recording system (Coulbourn Instruments, Allentown, PA) was connected to a personal computer that ran the control software (SuperLab, BioPac, Goleta, CA). Electrode impedances were held to 10 KOhms and the raw electromyography (EMG) signal was amplified and digitally sampled at 1000 Hz using AcqKnowledge software (Biopac, Goleta, CA). Sampling started immediately before the experimental block was started and lasted until the end of it (continuous recording).

Eyeblink EMG responses were digitally integrated (low-pass filtered between 1.0 and 500 Hz), rectified and smoothed digitally using AcqKnowledge software (Biopac, Goleta, CA). All trials were visually inspected for excessive noise in the EMG signal and for any software malfunctions. Startle responses were defined as the change from baseline to highest amplitude in microvolt within 20–200 milliseconds (ms) after probe onset using a computer peak amplitude detection algorithm of the AcqKnowledge software system. Trials were considered invalid and rejected if 1) a response onset was less than 21 ms; or 2) a response peak was greater than 200 ms after probe onset. The test-retest reliability of baseline startle amplitudes and percent PPI (as examined and described above) has been shown to be high in a study of 9 − 12 year old boys (intra-class correlation coefficients of 0.94 and 0.90, respectively in PPI sessions separated by a week (Hawk et al., 2002).

2.6. Statistical analyses

2.6.1. General analytical methods

Of the 415 children who participated in the PPI sub-study, 70 children were excluded from the final analysis due to software malfunctions during signal recording. Among the remaining 345 children, 364 trials (8.8 % of all trials) were excluded because the response onset was less than 21 ms and 130 trials (3.1 % of all trials) were excluded because the response peak was greater than 200 ms after probe onset. Of the 345 remaining children, 279 children had complete information on maternal cortical bone lead measurements. Our final study population was restricted to 279 children who participated in the PPI sub-study, were not missing maternal cortical bone lead measurements, did not experience EMG or probe delivery software malfunctions during the PPI experiment, and had valid trial responses.

Negative estimates of bone lead concentrations may occur for lead values close to zero; we used all point estimates without imposing a minimum detectable limit and excluded participants with uncertainty estimates for cortical bone lead values $\geq 10\ \mu g/g$ as a standard quality-control procedure (Wright et al., 2003). Use of all point estimates without imposing a minimum detectable limit has been identified as the most appropriate method for using these values in epidemiologic studies (Kim et al., 1995; Korrick et al., 2002).

Historically, several methods have been used to quantify PPI; the preferred methods are to quantify PPI as a "proportion of the difference from the control" or as a "proportion of control", as these methods are least affected by differences in baseline acoustic startle responses (Blumenthal et al., 2004). PPI quantified as a "proportion of the difference from the control" is a relative difference calculated as:

$$\frac{\textbf{\textit{average magnitude X}} - \textbf{\textit{average magnitude ITI}}}{\textbf{\textit{average magnitude ITI}}}$$

where average magnitude *X* is the average magnitude of responses to probes during pre-pulse tones and average magnitude ITI is the average magnitude of probe responses during the ITI. For regression methods, the substantial right skewness of PPI necessitates a transformation of our PPI variable. An appropriate solution for a linear regression framework would be to take the natural log of PPI + some constant *C* to avoid zero or negative values (Howell, 2007).

Thus, using C = 1, we defined a transformed PPI as:

$$\text{PPI}^{*}=\ln\left(\frac{\textbf{\textit{average magnitude X}}}{\textbf{\textit{average magnitude ITI}}}\right)$$

This new transformation is simply the natural log of the ratio of PPI quantified as a "proportion of control". In a linear regression framework with PPI* as the dependent variable, a negative beta coefficient value for an independent variable indicates increased PPI (more inhibition by the pre-pulse tone) while a positive beta coefficient value indicates reduced PPI (less inhibition by the pre-pulse tone). Henceforth, when we refer to PPI, we are referring to the log-transformed "proportion of control". Individual PPI scores were collapsed into target and non-target scores by stimulus onset asynchrony (SOA) for each participant. An overall PPI analysis was performed using a 2 × 2 mixed ANOVA (Condition: Attended vs. Ignored x SOA: 120 ms vs. 240 ms). We calculated descriptive statistics and examined distributions for all variables of interest.

2.6.2. Causal inference

Causal inference involves the comparison of two potential outcomes. Ideally, for each person in the population, we would want to compare what would have happened had that person been exposed to exposure *e*, compared to what would have happened had the person been exposed to an alternative exposure *e'* (Hernán et al., 2008; Schwartz et al., 2018). The intra-person contrast of these two outcomes, averaged over the entire population, would then constitute the average causal effect of treatment on the outcome. In practice, only one outcome for an individual is observed, corresponding to the exposure that individual had indeed experienced. The unobserved outcome is defined as "counter to fact" or *counterfactual* and needs to be estimated. Although estimating the counterfactual outcome is unfeasible at the individual level, causal methods aim to provide averaged estimates for counterfactual outcomes at the population level (Hernán et al., 2008; Schwartz et al., 2018).

Randomized trials are often lauded as the gold standard because when designed properly, they allow for the estimation of causal effects. In the setting of an ideal randomized trial (i.e. no loss to follow-up, full adherence to a well-defined assigned treatment, and double blind

assignment), the randomized treatment groups would have similar distributions of measured and unmeasured covariates and the only difference between them would be their treatment assignment (Hernán and Robins, 2018). Since the study groups are comparable (also defined as exchangeable) in every way but their assigned treatment, any difference in the average outcome between the groups could only be *caused* by the differences in the treatments across the groups. In this setting, any effect estimates we obtain from statistical models evaluating the association between treatment and outcome would have a causal interpretation.

Randomized trials are often unfeasible or unethical to execute, particularly in environmental epidemiology; hence, researchers must rely on other approaches (such as well-designed observational studies) to assess causal effects, being explicit about any assumptions that are made. To endow our estimates with a causal interpretation in the context of this study, we emulated a conditionally randomized trial under the three main conditions necessary for causal inference: consistency, exchangeability, and positivity. Our causal contrast of interest is the average PPI value that would have been observed if all children had been assigned to receive the mean prenatal lead value for the study population (A = 0) vs. the average PPI value that would have been observed if all children had been assigned to a standard deviation increase from the mean prenatal lead value (A = 1).

2.6.2.1. Assumptions of consistency, exchangeability, and positivity

2.6.2.1.1. Consistency. For our research question, consistency means that the average counterfactual PPI value that would have been observed if all children in the study population had been exposed to the mean prenatal lead, equals their observed average PPI value if they had indeed been exposed to the mean prenatal lead value. The consistency assumption additionally implies that the values of prenatal lead under comparison correspond to well-defined interventions. In other words, all components of treatment assignment that could impact the counterfactual outcomes have been specified. In our observational setting, maternal bone lead accumulation could occur through multiple exposure routes, such as from leaded paint in the home or from occupational settings. If the mechanisms through which bone lead accumulation occur have differential effects on PPI, the intervention becomes ill-defined, as the causal effect is now dependent on the specific mechanism through which prenatal lead exposure occurred. Although there exists several mechanisms through which maternal bone lead accumulation occurs, the effects of bone lead exposure on the child in utero is expected to depend on the accumulated bone lead concentration itself, and not on the underlying mechanisms leading to accumulation (Silbergeld, 1991). This assumption is known as *treatment-variation irrelevance* (Hernán and Robins, 2018).

2.6.2.1.2. Exchangeability. Exchangeability is assessed in terms of exchangeability for treatment, and exchangeability for censoring. In the present study, conditional exchangeability for treatment means that the probability of study participants to be exposed to a given value of prenatal lead depends only on the measured covariates (Hernán and Robins, 2018). Thus, we assume that after adjusting for relevant confounders, the two groups are exchangeable (i.e., no unmeasured confounding), except for their exposure level. Conditional exchangeability for censoring means that conditional on the measured covariates, those who participated in the PPI sub-study would have had the same PPI values as those who did not participate in the PPI sub-study, had they in fact participated.

2.6.2.1.3. Positivity. The positivity condition means that there is a non-zero, positive probability of being assigned to each treatment level in each stratum of the covariates. This condition is necessary to compute an average causal effect, as there must necessarily be exposed and unexposed individuals. In an ideal, marginally randomized trial with a binary treatment, the probability of being assigned treatment (Pr [A = 1]) and not being assigned treatment (1- Pr [A = 0]) are both positive by design, and therefore rarely evaluated as a condition for ideal randomized trials (Hernán and Robins, 2018). In an observational setting, positivity is not always expected to hold. Therefore, to acquire unbiased estimates in our models, we must assume, conditional on the covariates in our model, that each child has a non-zero probability of having any prenatal lead exposure.

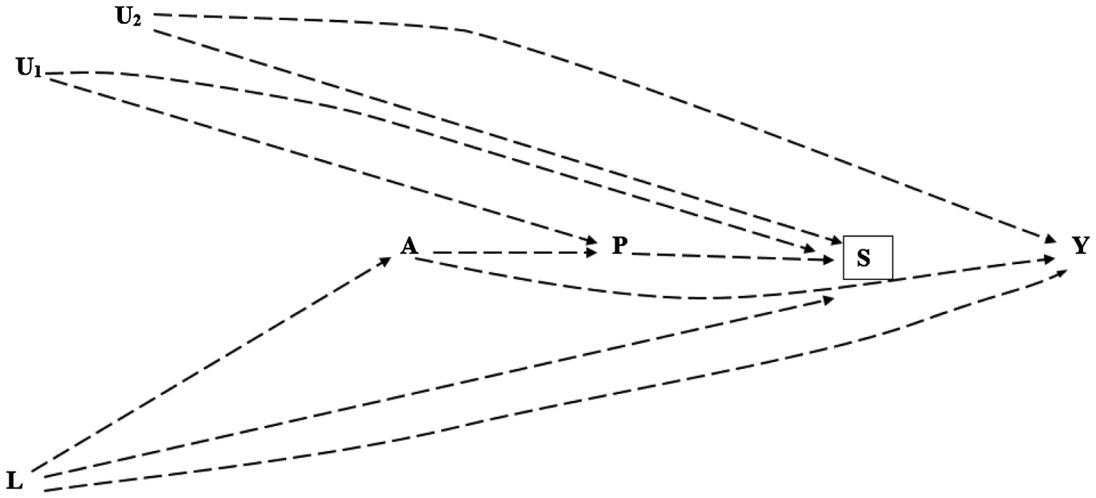
2.6.3. Assessing confounding and selection bias

To assess confounding, we considered the baseline covariates maternal marital status, maternal age, maternal education, maternal IQ, SES, and smoking during pregnancy. These covariates have been highlighted in previous studies as typical confounders of lead and neurodevelopmental disorders in children (Braun et al., 2017; Lanphear et al., 2005). We made decisions regarding confounding adjustment for these variables by using directed acyclic graphs (DAGs), causal structures that encode statistical relationships between important variables and the exposure and outcome of interest (Hernán et al., 2004). The statistical relationships encoded in our DAG were additionally informed by multiple imputation random lasso (MIRL), a variable selection algorithm that identified maternal education, total breastfeeding months, birthweight, child's age at PPI experiment, and child's sex as predictors of PPI in our study population (Liu et al., 2016; Kponee-Shovein et al., 2019). We used MIRL to identify predictors of PPI because PPI has typically been studied in cross-sectional, clinical studies. Given those settings, there is limited information on predictors of PPI that could inform confounding adjustment in epidemiological settings.

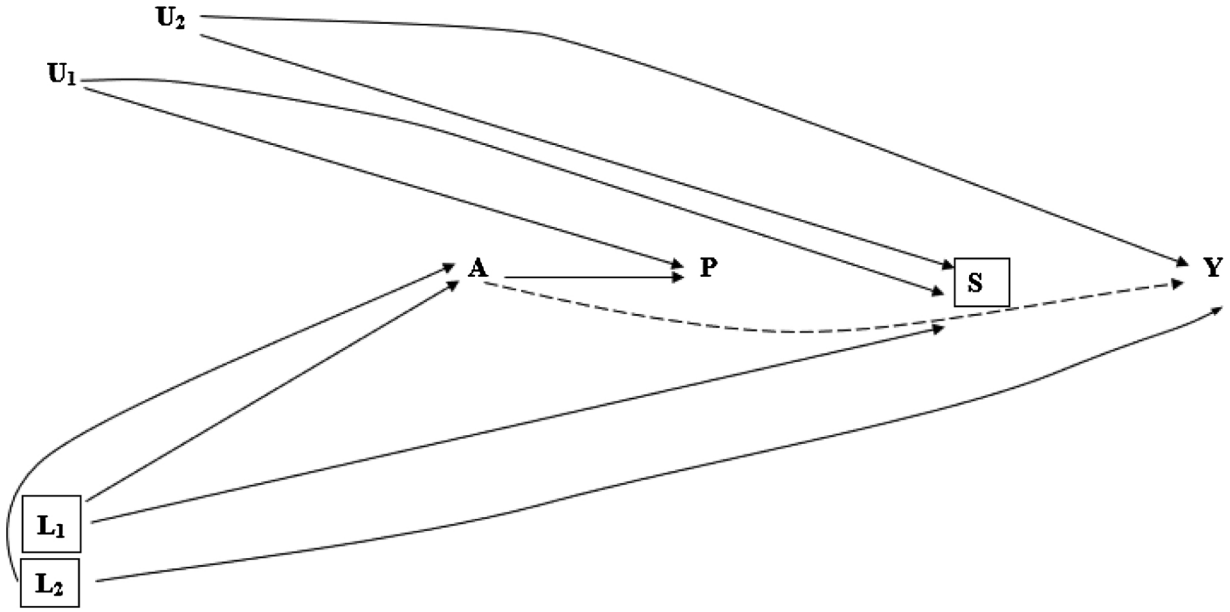
To assess selection bias, we compared distributions of prenatal lead concentrations between participants in our PPI sub-study and non-participants from the ELEMENT cohort. We regressed participation in the PPI sub-study on prenatal lead (using a linear and quadratic term) in a logistic regression model to assess if prenatal lead was associated with participation. We used logistic regression models to assess if covariates of interest were associated with participation in the PPI sub-study and with our PPI outcome using linear and quadratic terms. We also evaluated whether any post baseline variables predicted by prenatal lead were associated with both participation in the PPI sub-study and PPI using linear and quadratic terms. Finally, we examined the functional relationship between the variables included in our final model and PPI and added quadratic terms when suggested by the data.

The results from our analyses regarding confounding and selection bias are presented in Fig. 1. The first DAG represents our *a priori* assumptions about the statistical relationships present in our study based on prior studies on lead and neurodevelopmental disorders. The second DAG represents the statistical relationships informed by our data. Of the abovementioned potential confounders, only maternal education was identified as a confounder, therefore, we adjusted for maternal education to estimate the unbiased effect of prenatal lead on PPI. Of the covariates, linear maternal IQ ($p = 0.02$) was associated with selection into the PPI sub-study, and our statistical model suggested a quadratic relationship between maternal IQ and selection into the present study ($p = 0.03$). Due to the known association between prenatal lead and maternal IQ and *a priori* assumptions, we adjusted for maternal IQ and included a quadratic term.

In our study population, as evidenced by null effect estimates and *p*-values, linear prenatal lead ($p = 0.78$) and quadratic prenatal lead ($p = 0.98$) was not associated with selection into the present sub-study and we could find no measured post baseline variables predicted by prenatal lead that were associated with selection. As such, under the assumption of no unmeasured confounding (an assumption necessary for estimating causal effects in all observational studies), conditioning on maternal IQ and maternal education was sufficient to ensure exchangeability for treatment and selection in our study population. We additionally conditioned on child's sex and birthweight, two predictors of PPI not associated with prenatal lead, to enhance precision in our effect estimates (Schisterman et al., 2009). Child's age at PPI experiment, another predictor of PPI, was also conditioned on to account for the differential follow-up times of the three different ELEMENT cohorts



A: Exposure prenatal tibia lead
P: Vector of post baseline variables potentially predicted by prenatal tibia lead
S: Selection into the PPI sub-study
Y: Outcome PPI
L: Vector of potential confounders maternal age, maternal IQ, maternal education, maternal smoking during pregnancy, maternal marital status, SES level, and child's sex that may also be associated with selection into the PPI sub-study.
U1: Vector of unmeasured covariates potentially associated with the vector of covariates *P* and selection into the PPI sub-study
U2: Vector of unmeasured covariates potentially associated with selection into the PPI sub-study and PPI



A: Exposure prenatal tibia lead
P: The post baseline variable cumulative child blood lead
S: Selection into the PPI sub-study
Y: Outcome PPI
L1: Maternal IQ
L2: Maternal education years
U1: Vector of unmeasured covariates potentially associated with cumulative child blood lead and selection into the PPI sub-study
U2: Vector of unmeasured covariates potentially associated with selection in the PPI sub-study and PPI

Fig. 1. Directed Acyclic Graphs for the Total Effect of Prenatal Lead on PPI.

and to enhance precision. Based on previous literature (H Hu et al., 1995), we considered possible interactions between prenatal lead and maternal age, sex, and child's age at PPI test. These interaction terms were not statistically significant and were excluded from final models. Our final models for the primary analysis of the effect of prenatal lead on PPI included the covariates maternal education, maternal IQ, child's age at PPI sub-study, child's sex, and birthweight.

2.6.4. Models and estimations

We estimated the effect of prenatal lead on PPI using three methods:

Table 1
Characteristics of Participants and Non-participants from the ELEMENT Cohort.

	Present Study Participants (N = 279)		Non-participantsfrom the ELEMENT Cohort (N = 1819)		
Characteristics	n	Mean ± SD or n (%)	n	% Missing	Mean ± SD or n (%)
Age at PPI Testing (years)	279	13.3 ± 2.4	N/A	N/A	N/A
Sex, female	279	169 (49.0 %)	1617	11.1%	769 (47.5 %)
Gestational age (weeks)	279	39.0 ± 1.3	1606	11.7%	38.9 ± 1.6
Birthweight (kilograms)	279	3.1 ± 0.4	1621	10.9%	3.1 ± 0.5
Breastfeeding length (months)	279	8.8 ± 7.1	1354	25.6%	7.8 ± 6.3
Cumulative venous blood lead (μg/dL)[a]	279	6.2 ± 2.8	1399	2.2%	5.9 ± 3.2
Maternal tibia lead (μg/g)	279	9.3 ± 10.0	1150	36.8%	9.0 ± 9.9
Maternal patella lead (μg/g)	271	13.2 ± 12.4	1391	23.5%	11.0 ± 12.6
Maternal age (years)	279	26.1 ± 5.4	1809	0.5%	25.6 ± 5.3
Maternal education (years)	279	10.5 ± 3.1	1799	1.1%	10.3 ± 3.1
Maternal IQ	268	90.3 ± 20.6	1153	36.6%	87.5 ± 23.1
Maternal status, married	279	205 (73.5 %)	1807	0.7%	1240 (68.6 %)
Maternal parity	279	2.0 ± 1.1	1819	0.0%	2.0 ± 1.1
Prenatal smoking, ever	277	12 (4.3 %)	1798	1.2%	86 (4.8 %)
Paternal education (years)	262	10.5 ± 3.6	1634	10.2%	10.4 ± 3.5
SES index level[b]	271	8.6 ± 3.4	485	11.5%	8.5 ± 3.3

Abbreviations: SES, socioeconomic status; N/A, not available.
Note: N/A indicates variables that were measured only at the PPI experiment.
[a]: Cumulative blood lead represents the average of cord blood lead and venous blood lead measurements collected at 1, 2, 3, 4, and 5 years of age. These measurements were collected from 1710 children from the ELEMENT cohort.
[b]: SES index level was assessed only at the initial follow-up visit in 827 participants.

ordinary least squares (OLS) regression, the parametric g-formula, and a marginal structural model (MSM). The approaches for each method are explained in detail below.

2.6.4.1. Traditional least squares regression. We regressed PPI on prenatal lead and our final covariates in an OLS regression model, and used a robust variance estimation to obtain 95 % confidence intervals. Under the assumptions of consistency, exchangeability, and positivity, the counterfactual mean PPI outcomes are equal to the corresponding mean PPI outcomes in the data for our contrasts of interest (Hernán and Robins, 2018). Because we assumed that there was no effect modification, our slope estimate of β_1 is an estimate of both the conditional and marginal effect of prenatal lead on PPI (Hernán et al., 2004). If our assumption regarding effect modification is incorrect, our model is misspecified and our estimates are invalid. Methods such as the parametric g-formula and MSMs allow us to obtain valid effect estimates without requiring the additional assumption of additivity.

2.6.4.2. The parametric g-formula. The g-formula is a generalization of standardization that estimates the standardized outcome distributions using exposure and confounder specific estimates of the outcome distribution, in essence standardizing the mean outcomes to the distribution of the confounders (Hernán and Robins, 2018). In the case of few binary confounders, non-parametric methods can be used to obtain effect estimates. Since our current research question required adjusting for continuous covariates such that computing the standardized means non-parametrically was unfeasible, we used parametric regression modeling to estimate the standardized means via the parametric g-formula.

To estimate the effect of prenatal lead on PPI using the parametric g-formula, we used the three-step algorithm for the parametric g-formula outlined by Hernán et al. (Hernán and Robins, 2018). We used non-parametric bootstrap sampling with 10,000 samples to obtain percentile-based 95 % confidence intervals.

2.6.4.3. Marginal structural models. MSMs have been well described as causal models for estimating causal effects from observational data. The parameters of an MSM can be consistently estimated using inverse-probability-weighted estimators for treatment (Robins et al., 2000). We first used modeling approaches to create inverse-probability-of-treatment weighted estimators.

We regressed prenatal lead exposure on our covariates of interest in a linear regression model assuming normally distributed residuals. The standardized residuals from the model were used to evaluate the normal probability density function at the value of the residual to obtain the individual weights. Because weighting techniques for continuous exposures can lead to imprecise estimates, the precision of the weights can be improved by "stabilizing" the weights and thereby reducing the variability of the weights (Robins et al., 2000; VanderWeele et al., 2011). We stabilized the weights from our linear regression model by the normal probability density function of prenatal lead.

Finally, we fit a MSM for the expected counterfactual mean PPI outcome by regressing our observed PPI outcome on prenatal lead and weighting each participant by the stabilized inverse-probability-of-treatment weight. We used robust variance estimation to obtain 95 % confidence intervals to account for the sampling error in the estimation of the stabilized weights (Hernán and Robins, 2018; Robins et al., 2000).

2.6.5. Sensitivity analyses

We assessed the robustness of our estimate to additional adjustments for other confounders typically adjusted for in studies of lead and neurodevelopmental disorders (Braun et al., 2017; Huang et al., 2015; Lanphear et al., 2005). Specifically, we additionally adjusted for maternal marital status, maternal age, SES index level, and smoking during pregnancy using the abovementioned modeling approaches.

To confirm the use of the appropriate bone lead metric for our question of interest, we examined the relationship between prenatal trabecular bone lead and PPI using the same set of covariates for the primary analysis and the three modeling approaches described above,

We conducted all analyses using R Statistical Software, version 3.2.4 (Foundation for Statistical Computing, Vienna, Austria) and SAS 9.4 (Cary, NC)

3. Results

3.1. Baseline characteristics

Table 1 shows the distribution of characteristics for participants in the present study and non-participants from the ELEMENT cohort. In general, there were no meaningful differences in baseline

characteristics between the two groups, with the exception of maternal IQ points which was marginally higher for participants in the present study than for non-participants (90.3 vs. 87.5; $p = 0.05$). Among participants in the present study, the mean age at testing for children was 13.3 years and about half of the children were female. The average total education years was 10.5 years for mothers and fathers and only 4.3 % mothers reported smoking during pregnancy. Mean prenatal lead was 9.3 μg/g with a standard deviation of 10.0 μg/g of bone mineral density.

3.2. PPI task performance

The PPI task performance, evaluated by how well the 279 children identified their assigned target tone and the longer tones, was satisfactory. Among all responders, 65.6 % gave completely correct counts, 19.7 % gave partially correct counts and 14.7 % gave counts that were completely incorrect. Overall, the results of the task performance suggested that the participants understood and were able to appropriately perform the tasks assigned during the experiment

3.3. Overall PPI analysis

Fig. 2 displays the overall pattern of PPI for the attended and ignored conditions for SOAs of 120 ms and 240 ms. Consistent with Fig. 2, the 2 × 2 mixed ANOVA (SOA: 120 ms vs. 240 ms vs. Condition: Attend vs. Ignore) revealed a marginally significant main effect of SOA ($F(1, 267) = 2.1$, $p = 0.15$), no significant main effect of condition ($F(1, 267) = 0.7$, $p = 0.42$), and no significant SOA x condition interaction ($F(1, 195) = 0.0$, $p = 0.89$). In the absence of an interaction, we combined PPI results across condition and SOA for all subsequent analyses.

3.4. Primary analysis for the effect of prenatal lead on PPI

Table 2 presents results for the effect of prenatal lead on PPI using OLS regression, the parametric g-formula, and a MSM. The second and third columns provide estimated mean PPI values had all children been assigned the mean prenatal lead value of 9.3 μg/g versus had all children been assigned a one standard deviation increase (10.0 μg/g) to yield a prenatal lead value of 19.3 μg/g. Columns four and five provide slope estimates and percent changes for this causal contrast using the three modeling approaches. The slope estimates and 95 % confidence intervals were consistent across the three methods and indicate statistically significant effects of prenatal lead on PPI. Specifically, for the parametric g-formula, intervening to increase prenatal lead exposure by a standard deviation would result in a 0.2-point mean decrease in PPI (95 % CI: 0.1, 0.3). Based on our operationalization of PPI as a log transformed ratio, this estimate equates to a 19.0 % (95 % CI: 5.4 %, 34.3 %) decrease in PPI in our study population.

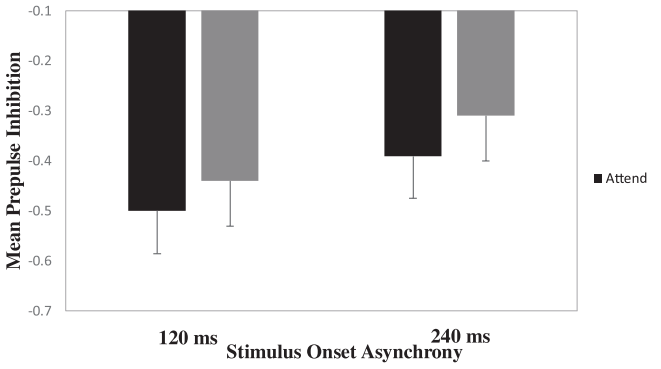


Fig. 2. Mean PPI for Attended and Ignored Task Conditions at 120 ms and 240 ms Stimulus Onset Asynchronies.
Note: Error bars indicate the standard error of the means

3.5. Sensitivity analyses

Adjusting for all baseline covariates typically considered confounders in studies of lead and neurodevelopmental disorders yielded similar effect estimates, although the estimates were slightly attenuated. For the parametric g-formula, intervening to increase prenatal lead exposure by a standard deviation would result in a 0.2-point mean decrease in PPI (95 % CI: 0.0, 0.3). This estimate equates to a 17.7 % (95 % CI: 2.5 %, 35.1 %) decrease in PPI in our study population (Table 3). When we examined the relationship between prenatal trabecular bone lead levels and PPI, we found a null association across all three modeling approaches (data not shown).

4. Discussion

In this present study, we used three different modeling approaches to estimate the causal effect of prenatal lead exposure on PPI in children. The results from our analyses suggest a statistically significant effect of prenatal lead exposure on PPI deficits in Mexican children 8–17 years of age. Our study is the first study to evaluate the relationship between lead and PPI in humans; only one prior study has investigated this relationship in rodents. Consistent with our results, the rodent study found mildly impaired PPI of the acoustic startle reflex in offspring of rats that had been exposed to lead (Ferguson et al., 1998).

Epidemiological studies have provided an abundance of evidence that prenatal lead exposure is associated with disruptions in neuropsychiatric functions (Baghurst et al., 1992; Huang et al., 2015; Lanphear et al., 2005; Senut et al., 2012). Consistent with studies of lead and neurodevelopmental disorders, our findings suggest that prenatal lead causes deficits in PPI. PPI deficits have been associated with a number of neurodevelopmental disorders in children and adolescents (Takahashi et al., 2011). Furthermore, the 19 % decrease in PPI for a standard deviation increase from the mean prenatal lead value observed in this study is similar in magnitude to clinical studies on schizophrenia, which have observed PPI impairments in patients with schizophrenia as compared to controls, with percent decreases in PPI ranging from approximately 16%–20% in these studies (Csomor et al., 2009; Mena et al., 2016). The consistency of findings from this study with previous clinical studies on PPI impairments and neurodevelopmental disorders provide additional support for the potential utility of PPI as an objective biomarker of neurotoxicity.

While previous studies on PPI impairments have been cross-sectional in nature, our study is the first prospective epidemiological study to examine this relationship. Our results provide some support for the use of PPI as an objective metric for the detection of early or subclinical neurological deficits in children exposed to prenatal lead. Additionally, given that PPI is a phenomenon with a well-defined neural basis, it may prove more objective than other measures (e.g., behavioral ratings) of neurodevelopmental disorders in children. In addition, deficits in PPI may be an earlier indicator of risk for later neurodevelopmental disorders, and therefore an earlier marker of the effects of environmental toxicants. Although impaired PPI has been observed in a range of neurodevelopmental disorders in human subject studies, a longitudinal study to assess whether PPI predicts later development of such disorders has not been done. Future studies that elucidate on the prospective association between PPI and neurodevelopmental disorders will be needed to inform relevant interventions.

The classic neural circuitry involved in the modification of the acoustic startle reflex via PPI is primarily localized in the brain stem, although recent studies also suggest the involvement of the forebrain regions in the regulation of sensorimotor gating (Rodrigues et al., 2017). Several studies have suggested that lead disrupts evoked potentials mediated by neurons in the brain stem (Alvarenga et al., 2015). Animal model studies have provided additional insight on mechanisms underlying neurotoxicity from prenatal lead exposure (Verina et al., 2007; White et al., 2007). These studies suggest that exposure to lead

Table 2
Slope Estimates[a] for the Total Effect of Prenatal Lead on PPI.

Modeling Strategy	Mean PPI for Prenatal lead at 9.3 μg/g[b] (95% CI)	Mean PPI for Prenatal lead at 19.3 μg/g[c] (95% CI)	Slope estimate (95% CI)	Percent change[d](%) (95% CI)
Crude model	−0.2 (−0.4, −0.1)	−0.1 (−0.2, 0.1)	0.2 (0.0, 0.3)	18.6% (4.3%, 35.0%)
Multivariable OLS model[e]	−0.2 (−0.4, −0.1)	−0.1 (−0.2, 0.1)	0.2 (0.1, 0.3)	19.0% (5.4%, 35.7%)
Parametric g-formula	−0.2 (−0.4, −0.1)	−0.1 (−0.3, 0.1)	0.2(0.1, 0.3)	19.0% (5.4%, 34.3%)
Marginal structural model	−0.2 (−0.4, −0.1)	−0.0 (−0.2, 0.2)	0.2 (0.1, 0.3)	19.0% (6.0%, 35.1%)

Abbreviations: PPI, prepulse inhibition; OLS, ordinary least squares; CI, confidence intervals.
[a]Adjusted estimates are based on adjustments for maternal education, maternal IQ, birthweight, child's sex, and child's age at PPI experiment. All estimates are rounded to the tenth place.
[b]The mean prenatal lead value represents the average cortical bone lead measurements for the study population.
[c]The mean prenatal lead at 19.3μg/g represents the average cortical bone lead value for the study population plus the standard deviation (10.0 μg/g) of the average cortical bone lead value for the study population.
[d]The percent change for each modeling strategy was calculated as ($e^{(\text{slope estimate})}$-1)*100.
[e]All other covariates in the model were set to their mean values.

Table 3
Sensitivity Analysis: Slope Estimates[a] for the Total Effect of Prenatal Lead on PPI.

Modeling Strategy	Mean PPI for Prenatal lead at 9.33 μg/g[b] (95% CI)	Mean PPI for Prenatal lead at 19.33 μg/g[c] (95% CI)	Slope estimate (95% CI)	Percent change[d] (%) (95% CI)
Crude model	−0.2 (−0.4, −0.1)	−0.1 (−0.2, 0.1)	0.2 (0.0, 0.3)	18.6% (4.3%, 35.0%)
Multivariable OLS model[e]	−1.4 (−2.8, 0.0)	−1.2 (−2.6, 0.2)	0.2 (0.0, 0.3)	17.8% (2.5%, 35.3%)
Parametric g-formula	−0.2 (−0.4, −0.1)	−0.1 (−0.3, 0.1)	0.2 (0.0, 0.3)	17.7% (2.5%, 35.1%)
Marginal structural model	−0.2 (−0.4, −0.1)	−0.1 (−0.3, 0.2)	0.2 (0.0, 0.3)	17.5% (2.4%, 34.7%)

Abbreviations: PPI, prepulse inhibition; OLS, ordinary least squares; CI, confidence interval; SES, socio-economic status.
[a]Adjusted estimates are based on adjustments for maternal education, maternal IQ, birthweight, child's sex, child's age at PPI experiment, maternal marital status, maternal age, SES index level, and prenatal smoking status. All estimates are rounded to the tenth place.
[b]The mean prenatal lead value represents the average cortical bone lead measurements for the study population.
[c]The mean prenatal lead at 19.3μg/g represents the average cortical bone lead value for the study population plus the standard deviation (10.0 μg/g) of the average cortical bone lead value for the study population.
[d]The percent change for each modeling strategy was calculated as ($e^{(\text{slope estimate})}$-1)*100.
[e]All other covariates in the model were set to their mean values.

can interfere with neurogenesis by inhibiting the proliferation, development, and survival of newly generated neurons in the developing fetus (White et al., 2007). Prenatal lead exposure inhibits neuronal development in the growing fetus and interferes with many biological system critical for regulating synaptic plasticity, such as the activity of protein phosphatases (Senut et al., 2012). Our results add to this line of research, providing evidence that prenatal lead exposure can disrupt neural systems that are responsible for regulating sensorimotor gating processes such as PPI. The long-term effects of these disruptions may subsequently lead to neuronal pathologies indicative of inadequate motor or sensory gating (Geyer, 2006).

Our models provide evidence of a causal effect of prenatal lead on PPI in children under the three main causal inference assumptions of exchangeability, consistency, and positivity. To evaluate the robustness of our estimates, we also adjusted for additional baseline covariates typically considered confounders in studies of lead and neurodevelopmental disorders. We found that these estimates were similar with estimates from our primary analysis across the three modeling strategies. This observation provides some evidence that PPI may be a more objective marker for adverse effects of toxicants on the brain than currently used metrics. Childhood neurodevelopmental disorders such as attention-deficit hyperactivity disorder (ADHD), autistic spectrum disorder (ASD), and early psychosis are typically evaluated using subjective measures that incorporate limitations such as informant biases (Emser et al., 2018; Sharma et al., 2018). Typical confounders that are adjusted for when evaluating the association between these disorders and environmental toxicants may be confounders for the environmental toxicant and the subjectivity of the metric, and not the underlying disorder itself. PPI is an automatic biological phenomenon that is measured using an EMG, suggesting that it's metric may be more objective than behavioral ratings.

In a sensitivity analysis, we evaluated the relationship between prenatal trabecular bone lead and PPI in children. We did not find an association between trabecular bone lead and PPI. This is expected because bone mineral density and bone turnover, especially resorption during pregnancy, occurs at a higher rate with trabecular bone than with cortical bone. This is because more trabecular bone surfaces are more readily available for turnover (Gulson et al., 2004) during pregnancy. Because trabecular bone lead was measured approximately 4 weeks postpartum in mothers, the trabecular bone lead measurements may not be a reliable proxy for prenatal bone lead measurements during pregnancy due to the higher turnover rate. As such, it is unsurprising that we observed a null association between trabecular bone lead and PPI.

Our assumptions that the results observed in our study population are not distorted by self-selection bias is supported by the null association observed between prenatal lead and selection into the present PPI sub-study. Despite the smaller study sample size, this reduction is not expected to systematically bias our findings, because selection bias results from conditioning on common effects (e.g., selection into the PPI sub-study) of an exposure lead and outcome under study (Hernán and Robins, 2018). Given that selection into the study is not predicted by

prenatal lead, there is some assurance that self-selection bias is not a concern in our study population. Given the ubiquity of lead in the environment, and the expected mobilization of prenatal bone lead during pregnancy at varying levels, we assumed that positivity held in our study. Given our small sample size of 279 mother-child pairs, there may exist violations of positivity due to a finite sample size, but this scenario is not expected to violate the assumptions for causal inference when parametric methods are employed. Parametric models smooth over random violations of positivity by borrowing information from other strata without random positivity violations.

The validity of our effect estimates is dependent on the additional assumptions of no model misspecification and measurement error. To protect against model misspecification, we examined the functional relationships of covariates of interest and included higher-order terms when suggested by the data. Our effect estimates are expected to be robust to measurement error due to our exclusion of maternal cortical bone lead measurements with uncertainty values greater than 10 μg/g as such high uncertainty suggests systematic error in ascertaining cortical bone lead concentrations due to excessive movement of the participant during measurement (H Hu et al., 1995). Additionally, our PPI values were restricted to children who had valid trial responses and who did not experience EMG malfunctions during the PPI experiment. Due to the randomness of the malfunctions, these exclusion of trials with EMG malfunctions should not result in any systematic biases, although, a consequence of this restriction is a loss in power.

Our estimate for the effects of prenatal lead on PPI were consistent across the three methods for all analyses. While the three methods employed different modeling strategies, all three methods made the same assumptions regarding exchangeability, positivity, and consistency, with the OLS regression model making an additional assumption about additivity. The consistency of our estimates across the three methods provide some evidence for the absence of serious model misspecification. A limitation of the OLS regression approach is that the average PPI value for our two exposure contrasts are conditional on the mean values for other covariates in the model and may be difficult to interpret meaningfully for policy purposes. A benefit to the parametric g-formula and the MSM is the ability to observe the marginal mean PPI outcomes for the two exposure contrasts. Using the parametric g-formula and the MSM, we can observe the mean PPI outcome had every child been exposed to a prenatal lead value of 9.3 μg/g versus if every child had been exposed to a prenatal lead value of 19.3 μg/g. As evidenced by our results, we observed a substantial reduction in PPI for a standard deviation increase from the mean prenatal lead value.

Our study is not without limitations. While our current study demonstrates that lead significantly impairs PPI, it is unclear if these impairments can be interpreted as a subclinical indicator of neurodevelopmental disorders. Although several cross-sectional studies have observed PPI deficits in children with various neurodevelopmental disorders (Takahashi et al., 2011), the limited control for confounding factors, small sample size, and the cross-sectional nature of these studies warrant additional investigations into the relationship between PPI and these disorders in larger, prospective studies. As with all observational studies, the assumption of no unmeasured confounding is an untestable hypothesis. Given the novelty of our study question, we cannot rule out the possibility of important confounders of lead and PPI that were not considered in our study. Finally, our estimates may not be generalizable to other populations with different distributions of lead, covariates, or effect modifiers not tested in the present study.

Nevertheless, the prospective design of our study enhances our ability to evaluate the temporal relationship between prenatal lead exposure and PPI. Additionally, the careful examination of the conditions necessary for endowing our effect estimates with a causal interpretation supports our assessment of prenatal lead as a determinant of PPI deficits instead of a correlate. The consistency of our study results with other human and animal model studies on lead and neurodevelopment highlight the potential value of PPI as an adjunct or screening tool for identifying children and adolescents at risk for various neurobehavioral disorders. Finally, the results from our study underscore the potential utility of PPI as a biologically relevant metric for assessing the relationship between environmental toxicants and neurodevelopmental disorders in epidemiological settings. Future research on the effects of other environmental toxicants on PPI and the prospective relationship between PPI and neurodevelopmental disorders will be valuable for environmental neurodevelopmental research.

5. Conclusion

Our study is the first to assess the effect of prenatal lead on PPI deficits, a neurological marker for various neurodevelopmental disorders, using traditional and novel methods in a cohort of children and adolescents from Mexico City. The results of our study suggest that prenatal lead exposure causes PPI deficits in children and adolescents. Our findings are consistent with results from epidemiological studies that have found associations between lead exposure and neurodevelopmental disorders in this population. Our results suggest that PPI may be a valuable adjunct screening tool for assessing neurotoxicant effects on the brain and highlight the need for prospective studies on the relationship between other environmental toxicants and PPI, and the relationship between PPI and a range of neurodevelopmental disorders in children and adolescents.

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Declaration of Competing Interest

The authors report no declarations of interest.

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