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Elevated lead levels in relation to low serum neuropeptide Y and adverse behavioral effects in preschool children with e-waste exposure

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HIGHLIGHTS

- High blood Pb, serum dopamine and substance P levels were found in the e-waste area.
- Low serum NPY levels level was found in the e-waste recycling area.
- Positive association between blood Pb and serum dopamine and substance P.
- There is a negative link between blood Pb level and serum NPY.
- NPY may mediate the link between Pb exposure and behavioral difficulties.

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ABSTRACT

As a neurotoxicant, lead (Pb) primarily affects central nervous system, and particularly impacts developing brain. This study explores the associations of blood Pb level and children's behavioral health. A total of 213 preschool children aged 3–7 years old were recruited from Guiyu (the e-waste-exposed area) and Haojiang (the reference area). The behavioral health of children was assessed using the 'behavioral symptoms' subscale of the Strengths and Difficulties Questionnaire (SDQ). Results showed that there was a significant difference in percent of children categorized as "at risk" between Guiyu (48.2%) and Haojiang (13.9%) ($p < 0.001$). The blood Pb level of children in Guiyu was significantly higher than those in Haojiang (median: 5.19 μg/dL vs. 3.42 μg/dL, $p < 0.001$). The serum Neuropeptide Y (NPY) was significantly lower in Guiyu children than those in Haojiang. Spearman correlation analyses demonstrated that blood Pb levels was negatively correlated with NPY ($r_s = -0.25$, $p < 0.001$), but positively correlated with behavioral symptom scores; while serum NPY levels were negatively associated with behavioral symptom scores. Behavioral symptom scores were higher in children with blood Pb level ≥5.00 μg/dL (high) than those with blood Pb level < 5.00 μg/dL (low). After adjusting for confounding factors, children with lower NPY levels were at higher risk of having behavioral difficulties. In conclusion, Pb exposure in e-waste-exposed areas may lead to decrease in serum NPY and increase in the risk of children's behavioral problems. In addition, NPY may mediate the association between Pb exposure and behavioral difficulties.

1. Introduction

With the rapid development of electronic industry, the innovation of global electronic products and the acceleration of electronic equipment or products upgrading in recent years, electronic

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waste (e-waste) have become the fastest growing garbage in the world (Zeng et al., 2020). E-waste refers to discarded electrical and electronic equipment after the end of its service life, whose abandoned components have contained potentially harmful contaminants such as heavy metals and persistent organic pollutants (Zeng et al., 2019). It was estimated that China generated the highest e-waste quantity (approximately 20% of global e-waste) both in Asia and in the world, and only 15% of e-waste has been documented to be collected and properly recycled (Zhang et al., 2012; Song and Li, 2014; Heacock et al., 2016; Zeng et al., 2016a,b; Priyadharsini and Singh, 2019). E-waste can be stored in a variety of environmental media including air, dust, soil, water, and sediment for a long time, which will inevitably cause environmental media pollution and pose a threat to the health of local residents (Robinson, 2009). Guiyu, a town located in southeast of China, is well known e-waste destination with informal and primitive recycling style in term of home-based workshops (Wu et al., 2010). Although a centralized industrial park has been recently established to recycle e-waste, the operation mode is rent workshop to individual merchants. Therefore, the improper and unsafe disposal activity is still exist companying with the process of e-waste collecting and recycling due to economic benefits in Guiyu (Alassali et al., 2020). It cannot be ignored that e-waste exposure and subsequently environmental pollutants have serious impaired human health.

Among the various environmental contaminants, especially heavy metals, lead (Pb) has attracted widespread attention by researchers for its characteristics of major and typical with higher exposure level compared with other areas over the world (Zeng et al., 2020; Zhang et al., 2020). It is well known that Pb is a non-degradable hazardous substance with cumulative toxicity. In addition, there is no safe exposure level to Pb, even at a very low level. Pb can enter the bloodstream quickly and easily through the inhalation, ingestion and skin adsorption in the form of fine particles, aerosols and their vapors (Nehru et al., 2001; Zeng et al., 2016a,b). Previous studies showed that Pb has been the most typical heavy metal in e-waste with high content in Guiyu (Wong et al., 2007; Xu et al., 2015; Zeng et al., 2020). In particular, Pb in the road dust of Guiyu is 330 and 106 times higher than non-e-waste sites located 8 and 30 km away, respectively (Leung et al., 2008). Preschool children are more likely to contact dust Pb because that they have special physical and behavioral characteristics when compared with adults. For instances, preschool children with lower height closer to the ground are more likely to inhale more dust Pb; preschool children with higher frequency of hand-to-mouth activity during playing outdoor are more likely to ingest more environmental medium Pb (Heacock et al., 2016; Zeng et al., 2020). Our previous studies demonstrated that preschool children living in Guiyu have significantly higher blood Pb levels than those from the reference areas (Huo et al., 2007; Liu et al., 2011, 2018; Xu et al., 2015; Zeng et al., 2016, 2017; Zeng et al., 2017a,b; Chen et al., 2019; Hou et al., 2020; Zhang et al., 2020). Percentages of preschool children with blood Pb levels ≥ 5 μg/dL (the recommended limit) were higher in Guiyu than those in the reference areas (Zheng et al., 2019; Hou et al., 2020; Zhang et al., 2020). This indicates that environmental and blood Pb exposure is still very serious in Guiyu and cause a considerable health burden to children and their families.

It is well known that children are at great risk of Pb poisoning which is not only impair peripheral nervous systems, but also damage the central nervous system (CNS) and the developing brain (Sanders et al., 2009). The key mechanism of Pb affecting the CNS is that it can impair and subsequently pass through the blood-brain barrier (BBB) partly for its ability to substitute for various polyvalent cations such as calcium (Ca^{2+}) and zinc (Zn^{2+}) ions (Garza et al., 2006; Wu et al., 2020). The biological process of competitive substitution of Pb^{2+} for Ca^{2+} or Zn^{2+} significantly impact metal transport, ionic conduction, signal transduction, energy metabolism, and genetic regulation. The flow and interaction of ions, molecules, and cells between blood and brain is precisely regulated by the BBB that is a structural and functional interface closely dominating the extracellular environment of brain (Friedman and Kaufer, 2015; Profaci et al., 2020). The impaired or dysfunction of blood brain barrier will certainly contribute to cognitive dysfunction, altered behavior, and neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease (Zenaro et al., 2017; Nation et al., 2019; Israelov et al., 2020). In addition, Pb exposure can cause acute or chronic symptoms such as mental retardation, learning disabilities, emotional problems, and behavioral disorders (Canfield et al., 2003; Liu et al., 2014a,b; Taylor et al., 2017; Freire et al., 2018). For an instance, elevated blood Pb was correlated with decrease in IQ and cognitive function even blood Pb level <5 μg/dL (Koller et al., 2004; Huang et al., 2012; Gump et al., 2017). Persistent behavioral problems have been shown to have a negative impact on children's intellectual, psychosocial, and academic development (Jansen et al., 2007). However, the effects of Pb outside CNS remain unclear. The neurotransmitters such as neuropeptide Y, substance P and dopamine are broadly expressed in the central and peripheral nervous system during childhood, and may function as a link between Pb and CNS, and further play a mediate role in Pb triggering behavioral problem (Hansel et al., 2001).

Neurotransmitters such as Neuropeptide Y (NPY) and Substance P (SP) involve in handling the emotion-eliciting information in the limbic system (Dalgleish, 2004). NPY is an important anxiolytic endogenous peptide being modulated by chronic stress, which is considered to play a very important role in emotion and stress coping (Heilig and Thorsell, 2002; Sah and Geracioti, 2013; Enman et al., 2015). In addition, it can induce regeneration of hippocampal neurons and participate in changes of synaptic plasticity. What is more, NPY neurons have been shown to interact with behavioral brain pathways and dopaminergic reward in the amygdala and nucleus accumbens, respectively (Dalgleish, 2004; Heilig, 2004; Morales-Medina et al., 2010). Substance P is highly expressed in hypothalamus, amygdaloid nucleus, and the periaqueductal gray, regulating emotion (Datar et al., 2004; Herpfer et al., 2007; Lorente et al., 2019). Elevated substance P levels are associated with increased inner tension and anxiety (Ebner and Singewald, 2006; Herpfer et al., 2007; Iftikhar et al., 2020). Previous studies demonstrated that the release of NPY and substance P have been proven to be activity-dependent, which can be modulated by N-methyl-D-aspartate (NMDA) receptor through the Ca^{2+} signaling pathway. Dopamine plays a crucial role in regulating behavior and emotions, and dopamine alteration lead to abnormal behaviors such as impulsivity and hyperactivity (Nieoullon and Coquerel, 2003; Strafella, 2019). Dysregulation of dopamine neurotransmission in dorsal striatal dopamine system is associated with the processing of negative human emotions (Badgaiyan, 2010; Chang et al., 2019). Persistent and developmental Pb exposure induces depressive behavior both in rats and children (Sciarillo et al., 1992; de Souza et al., 2005; Liu et al., 2014a,b).

Pb can enter the well-protected brain through damaging BBB for competing and substituting calcium and its roles in communication among neurons. In general, calcium enters the activated neuron and triggers the release of neurotransmitters such as neuropeptide Y, substance P and dopamine. Neurotransmitters participate in cascade reaction carrying neuronal signaling from the first neuro to the next nearby neuron until the signal reaching its target. Less Ca enter the neuron when competed with Pb, and subsequently weaker signal release from neurotransmitter to neuron. In addition, Pb also cause inappropriate neurotransmitter release in resting/

non-activated neurons and subsequently abnormal signals to later neurons. The above-mention process will lead to behavioral alterations. Long term Pb exposure increased immune-reactivities of anti-serotonin (anti-5HT) and tyrosine hydroxylase (anti-TH), and induced behavioral changes such as hyperactivity and anxiety in meriones (Bouyatas et al., 2019). Exposures to Pb are associated with increased repetitive behavior in mice, which linked with changed neurotransmission of dopamine (Chang et al., 2014). There are some studies investigating the effects of Pb on behavioral problems in animals and humans (Rice et al., 1996; Moreira et al., 2001; Bellinger et al., 2008; Liu et al., 2014a,b). Our previous study demonstrated that children exposed to Pb in an e-waste recycling area more like to have a higher blood Pb level and a lower cognitive and language scores when compared with the reference group (Liu et al., 2015). However, study on Pb-linked behavioral toxicity is inadequate in preschoolers from e-waste dismantling areas. Therefore, this study was designed to assess the adverse effects of Pb on behavioral health in e-waste-exposed preschool children.

2. Materials and methods

2.1. Study population

A total of 213 children (3–7 years old) from Guiyu (n = 112, the e-waste-exposed area) and Haojiang (n = 101, the reference area) which is located 31.6 km to the east of Guiyu. Both areas were similar in population, local customs and socioeconomic status. The big difference between the two areas is that e-waste pollution occurs only in Guiyu but not in Haojiang (Zeng et al., 2017a,b). Participants were recruited during the period of October to December 2017. The venous blood of the participants was collected by trained nurses. Meanwhile, a questionnaire was completed by the parents of each child, which covered individual details, behavioral habits, passive smoking conditions, family socioeconomic status and education levels of the parents.

2.2. Assessment of behavioral symptoms

Behavioral health of the children was assessed using the subscale "behavioral symptoms" of the Strengths and Difficulties Questionnaire (SDQ; available from www.sdqinfo.com). The standardized SDQ is an internationally applied and validated screening instrument, which includes five subscales: behavioral symptoms, conduct problems, hyperactivity-inattention, peer problems and prosocial behavior (Goodman et al., 2003). The original version was designed for parents of 4–16 years old children. In the present study, the age range of the participants was 3–6 years old. Five items of the behavioral symptoms subscale were completed by the parents to rate their child's behavioral health over the previous six months. Each of the five items was scored on a 3-point scale: 0 = not true; 1 = somewhat true; 2 = certainly true. The scores for the behavioral symptom subscale ranged from 0 to 10, with higher scores indicating more behavioral problems (Sioen et al., 2013; Philippat et al., 2017). All questionnaires were completed under the direction of well-trained researchers.

To investigate the prevalence rate of behavioral symptoms of preschool children in this study, we refer to the available Chinese normative data (Du et al., 2008). The Chinese cut-off points were used to designate children as normal (range 0–3), borderline (range 4) or abnormal (range 5–10) for the symptom score. The cut-off points originally presented by Goodman may result in imbalanced groups with very few participants in the abnormal group. Therefore, the classification of behavioral symptoms in children was categorized as normal (range 0–3) and at-risk (range 4–10) in this study (Holling et al., 2008). On the whole, the group classification mentioned above was used to explore associations with the other factors, while the children's behavioral symptom scores were used to investigate the difference between the two groups.

2.3. Measurement of blood Pb level

A total of 4 mL venous blood was collected from each fasting participant by well-skilled nurses and distributed in Pb-free tubes containing EDTA as an anticoagulant. The blood sample were stored at −80 °C until assay. To measure blood Pb level, 100 μL whole blood was digested in 900 μL 0.5% nitric acid, and the mixture was immediately shaken for 10 s and then stand and digest for 10 min. The blood Pb solution was centrifuged for 10 min at 2000 rpm after digestion to obtain supernatant for analysis. Finally, 400 mL solutions were put in the sample cup for blood Pb concentration measurement using graphite furnace atomic absorption spectrophotometry (Zeng et al., 2017a,b). Precision for blood Pb concentration was expressed as the relative standard deviation which was required to be less than 15%. Linearity for a five-point Pb calibration curve was good for blood Pb concentration ($r2 > 0.995$). The spiked recovery of blood Pb concentrations were generally within the 95–106%.

2.4. Determination of serum dopamine, substance P and NPY levels

The sample tube of blood was centrifuged at 1000 g for 15 min at 4 °C, then serum was obtained and stored at −80 °C until assay. Serum NPY levels were determined using an EIA system (EK-049-03, Phoenix Pharmaceuticals Inc., California, USA), as described previously (Sanchez-de-la-Torre et al., 2011). Assay sensitivity for NPY was 0.09 ng/mL. Measuring range for NPY was 0–100 ng/mL. Subsequently, dopamine serum levels were determined using a commercially available ELISA kit (DEE5500, Demeditec Diagnostics GmbH, Kiel, Germany), and serum substance P levels were determined with a Parameter™ ELISA kit (KGE007, R&D Systems Inc., Minneapolis, MN, USA). Assay sensitivities for dopamine and substance P were 6.6 pg/mL and 43.8 pg/mL, respectively. Measuring ranges for dopamine and substance P were 0.5–80 ng/mL and 39.0–2500 pg/mL, respectively.

2.5. Statistical analysis

Analyses of statistical data were performed with IBM SPSS22.0 and Graph Pad Prism 7.0 software. Descriptive statistics of continuous variables were expressed as median [interquartile range (IQR)] for abnormal distribution, or as mean ± standard deviation for normal distribution, and categorical data was presented as count and percentage. During analyses we deleted the unusual values and extremum to ensure comparability between groups. Differences between two groups were appropriately analyzed using Mann Whitney U, independent-sample t or chi-square tests for different types of data. Spearman's correlation analysis was used to evaluate potential confounding factors contributing to blood Pb level and behavioral symptom score. Confounders were chosen based on pre-existing knowledge, and included factors possibly related to both Pb exposure and children's behavioral health such as age, gender, maternal education levels, monthly household income, left-behind child, mother's work related to e-waste, residence as workplace, number of e-waste sites within 50 m of dwelling, and sucking/biting toys. In order to satisfy the assumption of normality, the blood Pb level was log-transformed by using the natural logarithm. After confounders were adjusted, multiple linear regression models were used to examine the associations between lnBPb and serum dopamine, substance P, and NPY. Binary logistic regression analysis was used to estimate the odds ratio for having an abnormal test score in relation to the Pb exposure and neurotransmitter levels. A *p*-value < 0.05 was considered statistically significant in two-tailed tests.

3. Results

3.1. Demographic characteristics of the study population

The demographic characteristics of the 213 participants are shown in Table 1. The average age of the children in the e-waste exposed group and the reference group was 4.73 ± 0.77 years and 4.87 ± 0.86 years, respectively. The average of age showed no significant difference between the two groups ($p > 0.05$). The blood Pb concentration of exposed children was 5.19 μg/dL, which was almost 1.5-times higher than the 3.42 μg/dL for reference children ($p < 0.001$). Furthermore, the percentage of blood Pb levels ≥5 μg/dL in the e-waste exposed group (54.5%) was significantly higher than that in the reference groups (9.9%) ($p < 0.001$). The percentage of children with behavioral difficulties from exposed group was higher than their peers from the reference group (48.2% vs. 13.9%, $p < 0.001$). There were significant differences between the two groups for mother's work involved in e-waste, family monthly income, and maternal education levels ($p < 0.05$).

3.2. Factors contributing to blood Pb levels and behavioral symptoms

Spearman correlation analysis was performed to evaluate whether certain factors were related to blood Pb levels and behavioral symptoms in preschool children (Table 2). The findings indicated that maternal education levels and monthly household income were negatively correlated with blood Pb concentrations. In other words, higher maternal education levels and monthly household income are the protective factors of Pb. However, residence as a workplace and number of e-waste sites within 50 m of dwelling were positively correlated with blood Pb concentrations. In addition, higher maternal education level and family monthly income was correlated with lower children's behavioral symptom scores. While sucking/biting toys, children left-behind, increased number of e-waste sites within 50 m of residence were correlated with higher behavioral symptom scores of children.

3.3. Differences in children's behavioral symptoms between the two groups

To analyze the behavioral health of preschool children in the exposed and reference groups, we measured scores using the 'behavioral symptoms' subscale of the SDQ. As shown in Fig. 1, the exposed group had a higher behavioral symptom score compared to the reference group (median 3.00 vs. median 2.00, $p < 0.001$). Furthermore, the behavioral symptom score was higher for both boys and girls living in the exposed group when compared to the children from the reference group (boys: median 2.50 vs. median 2.00, $p < 0.05$; girls: median 5.00 vs. median 2.00, $p < 0.001$, respectively).

Serum dopamine, substance P, NPY distributions and the relationships to blood Pb level.

We stratified by gender and analyzed the differences of neurotransmitter concentrations in serum between the exposed and reference areas (Fig. 2). Significant differences in all neurotransmitter levels were witnessed between the two groups ($p < 0.001$).

Table 1
Demographic characteristics of the study populations.

Characteristics	Exposed group (n = 112)	Reference group (n = 101)	Statistics	*P*-value
Age (mean ± SD, years) [n (%)]	4.73 ± 0.77	4.87 ± 0.86	t = 1.29	0.199
3 years	15 (13.4)	21 (20.8)		
4 years	59 (52.7)	31 (30.7)		
5 years	30 (26.8)	40 (39.6)		
6 years	8 (7.1)	9 (8.9)		
Gender [n (%)]				
Boys	60 (53.6)	53 (52.5)	$\chi^2 = 0.026$	0.891
Girls	52 (46.4)	48 (47.5)		
Blood Pb level (μg/dL) [median (IQR)]	5.19 (3.91,7.09)	3.42 (2.82,3.91)	Z = −7.442	<0.001
≥5 μg/dL [n (%)]	61 (54.5)	10 (9.9)	$\chi^2 = 47.460$	<0.001
<5 μg/dL [n (%)]	51 (45.5)	91 (90.1)		
Behavioral symptoms [n (%)]				
Normal	58 (51.8)	87 (86.1)	$\chi^2 = 28.838$	<0.001
At risk	54 (48.2)	14 (13.9)		
Left-behind child (yes/no)	(21/91)	(12/88)	$\chi^2 = 1.831$	0.190
Mother's work involved in e-waste (yes/no)	(6/105)	(0/101)	$\chi^2 = 5.618$	0.030
Sucking/biting toys [n (%)]				
None	81 (72.3)	76 (75.2)	$\chi^2 = 3.525$	0.318
Occasionally	30 (26.8)	23 (22.8)		
Often	0 (0.0)	2 (2.0)		
Always	1 (0.9)	0 (0.0)		
Family monthly income (CNY) [n (%)]				
<1500	4 (3.8)	1 (1.0)	$\chi^2 = 11.622$	0.020
1500−3000	18 (17.1)	12 (11.9)		
3000−4500	21 (20.0)	17 (16.8)		
4500−6000	27 (25.7)	15 (14.9)		
>6000	35 (33.3)	56 (55.4)		
Maternal education levels [n (%)]				
Primary school	19	2	$\chi^2 = 40.315$	<0.001
Middle school	60	30		
Vocational school	12	15		
High school	4	18		
College/university	16	36		

Values of $p < 0.05$ were considered statistically significant. IQR: interquartile range.

Table 2
Spearman correlation analysis of factors related to lnBPb and child's behavioral symptom score.

	Behavioral symptom score (r_s)	LnBPb (r_s)
Age	−0.131	−0.013
Gender	0.149*	−0.070
Sucking/biting toys	0.138*	0.114
Maternal education levels	−0.291***	−0.296***
Left-behind children	0.166*	0.109
Family monthly income	−0.156*	−0.195**
Mother's work involved in e-waste	0.010	0.112
Residence as workplace	0.110	0.234**
Number of e-waste sites within 50 m of dwelling	0.177*	0.213**

Note: LnBPb, ln-transformed blood Pb level. $p < 0.05$ was considered statistically significant. r_s, Spearman correlation coefficient. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

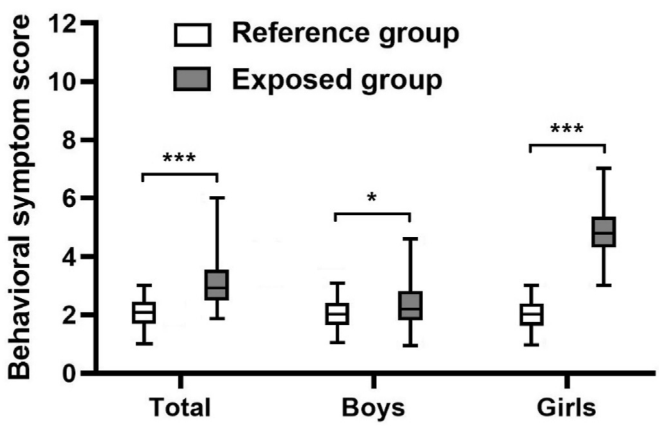

Fig. 1. Children's behavioral symptom score stratified by gender from the e-waste-exposed and reference groups. Exposed group: n = 112. Reference group: n = 101. Results are presented as the median (interquartile range). Data analysis by the Mann-Whitney U test. *$p < 0.05$. ***$p < 0.001$.

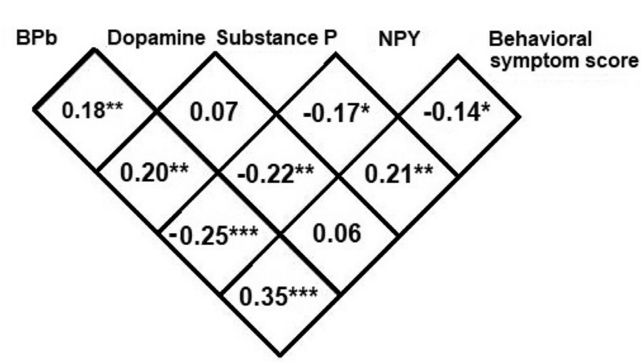

Fig. 3. Spearman rank correlations (r_s) of BPb, dopamine, substance P, NPY and behavioral symptom score. BPb, blood Pb level. *$p < 0.05$. **$p < 0.01$. ***$p < 0.001$.

Both boys and girls living in the exposed area had significantly higher dopamine and substance P levels than those living in the reference area. Conversely, the NPY concentrations in both boys and girls from the exposed area were significantly lower than in those in the reference area.

For the purpose of understanding the relationships between serum neurotransmitter concentrations and blood Pb level, we presented the association of neurotransmitter concentrations with Pb exposure (Fig. 3). Results showed that blood Pb level was positively correlated with dopamine, substance P, and negatively associated with NPY (both $p < 0.01$). In addition, we utilized different linear regression models (Table 3). Unadjusted regression analysis demonstrated that higher ln-transformed blood Pb levels were associated with lower NPY [B (95% CI) = −0.065 (−0.106, −0.024), $p < 0.01$], and with higher substance P [B (95% CI) = 0.083 (0.018, 0.148), $p < 0.05$]. However, after adjustment for age and gender, we only observed significant differences between lnBPb and NPY ($p < 0.01$). Further adjustment for maternal education levels, monthly household income, left-behind child, mother's work involved in e-waste, and sucking/biting toys, resulted in a slight decrease in relational degree [B (95% CI) = −0.052 (−0.095, −0.008), $p < 0.05$].

3.4. Relationship between Pb exposure and behavioral symptoms

Spearman correlation analysis results showed that blood Pb level was positive correlated with behavioral symptoms score

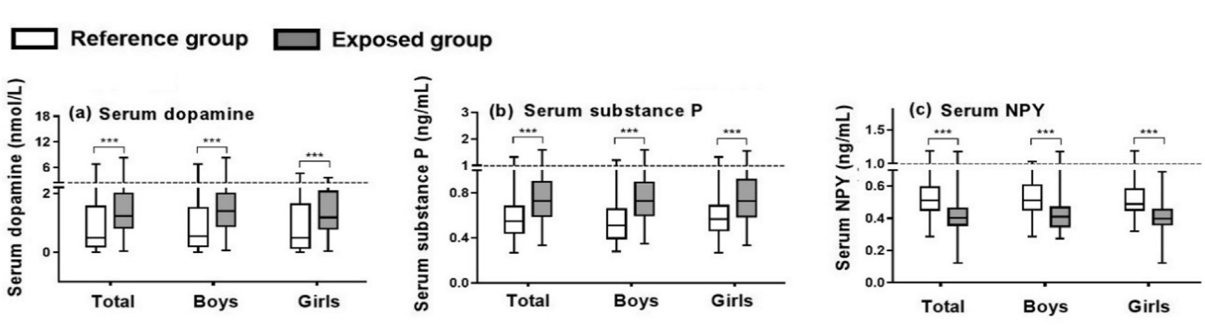

Fig. 2. Levels of serum dopamine, substance P and NPY stratified by gender from the e-waste-exposed and reference groups. Data analysis by the Mann-Whitney U test. ***$p < 0.001$.

Table 3
Multiple linear regression analysis for associations between lnBPb and serum dopamine, substance P, and NPY.

LnBPb	Dopamine		Substance P		NPY	
	B (95% CI)	β	B (95% CI)	β	B (95% CI)	β
Model 1	0.173 (−0.137, 0.483)	0.076	0.083 (0.018, 0.148) *	0.170	−0.065 (−0.106, −0.024) **	−0.210
Model 2	0.172 (−0.139, 0.483)	0.075	0.080 (0.015, 0.146)	0.164	−0.062 (−0.103, −0.021) **	−0.200
Model 3	0.094 (−0.231, 0.418)	0.041	0.046 (−0.022, 0.114)	0.096	−0.052 (−0.095, −0.008) *	−0.167

Model 1: unadjusted. Model 2: adjusted for gender, age. Model 3: adjusted for gender, age, maternal education levels, monthly household income, left-behind child, mother's work involved in e-waste, and sucking/biting toys. LnBPb, ln-transformed blood Pb level; B, unstandardized coefficient; CI, confidence interval; β, standardized coefficient.
*p < 0.05. **p < 0.01. ***p < 0.001.

($r_s = 0.35$, $p < 0.001$) (Fig. 3). Logistic regression analysis was used to evaluate the association between blood Pb and behavioral symptoms (Table 4). Univariate regression analysis showed that elevated blood Pb (LnBPb)were positively associated with behavioral problems [OR, 4.388; 95% CI (2.223, 8.661)]. After further adjustment for age, gender, maternal education levels, monthly household income, left-behind child, mother's work involved in e-waste, and sucking/biting toys, a similar trend was observed. In brief, Pb exposure was an independent risk factor for behavioral difficulties.

3.5. Association between related biomarkers and behavioral symptom scores

Spearman correlation analysis showed that the behavioral symptom score was positively correlated with substance P levels ($r_s = 0.21$, $p < 0.01$), and negatively associated with NPY levels ($r_s = -0.14$, $p < 0.05$) (Fig. 3). However, significant associations among dopamine levels and behavioral symptom scores were lost. Univariate regression analysis showed that behavioral problems were negatively associated with NPY levels [OR, 0.034; 95% CI (0.003, 0.399)] (Table 4), even after controlling for risk factors.

3.6. Effect of Pb exposure on neurotransmitter and behavioral difficulties

To further examine the effect of Pb exposure on neurotransmitter and behavioral symptoms, we defined children with a blood Pb level of more than or equal to 5 μg/dL as the high blood Pb level group, and children with a blood Pb level of less than 5 μg/dL was defined as the low blood Pb level group (Betts, 2012), and then compared the serum dopamine, substance P, NPY levels and the risk of behavioral difficulties between the two groups defined by the above given threshold of blood Pb level.

The median concentration of serum dopamine and substance P in children from the low blood Pb level group was lower than children from the high blood Pb level group (both $p < 0.05$) (Fig. 4). In contrast, the level of NPY in children from the low blood Pb level group (median: 0.47 ng/mL; range: 0.24 to 1.19) was higher than those from the high blood Pb level group (median: 0.41 ng/mL; range: 0.12 to 1.18) ($p < 0.01$). From the results obtained so far, it seems that the median of child's behavioral symptoms score from the low blood Pb level group was lower compared with those from the high blood Pb level group ($p < 0.001$) (Fig. 4).

4. Discussion

The present study explored the correlation between Pb exposure and the behavioral health in children aged 3- to 7- years old in an e-waste recycling area. The changes of three serum biomarkers of the behavioral disturbance were measured and the behavioral health status was evaluated using the SDQ in children from both e-waste and reference groups. Our results show that the average blood Pb level of children in Guiyu is 5.19 μg/dL, and about 54.5% Guiyu children with blood Pb level exceed 5 μg/dL. According to our previous studies, the percentages of blood Pb level ≥5 μg/dL of Guiyu children in 2007 (Huo et al., 2007), 2014 (Liu et al., 2014a,b), 2016 (Zeng et al., 2016a,b), and 2020 (Chen et al., 2020) were 91%, 83%, 66%, and 37%, respectively. These results indicate that there is a downward trend in blood Pb levels of Guiyu children over time, which may imply that the degree of Pb pollution are diminishing with the efforts of scientific workers and the promulgation and enforcement of national e-waste policies and legislation. Nonetheless, the blood Pb levels of Guiyu children is still higher than that of Haojiang. The average blood Pb concentration in this study is higher than 3.8 μg/dL in Uruguay children, 4.5 μg/dL in USA, and 4.94 μg/dL in children of northern China (Zhao et al., 2013; Kordas et al., 2018; Horton et al., 2019; Mielke et al., 2020). Moreover, we investigated multifarious related factors by a general questionnaire, including child behavioral habits, family socioeconomic status, and dwelling environment. The findings indicate that residence as workplace and increased number of e-waste sites within 50 m of dwelling are correlated with a higher blood Pb level. However, lower levels of maternal education and monthly family household income is correlated with elevated blood Pb level in children. In brief, the situation of Pb pollution in the e-waste exposed area is still serious, and its behavioral effects to children deserves public attention (Sanders et al., 2009; Liu et al., 2014a,b; Zeng et al., 2020).

Table 4
Binary logistic regression analysis for associations between risk of behavioral difficulties and lnBPb, serum dopamine, substance P, and NPY.

Risk of behavioral difficulties	OR[a] (95% CI)	OR[b] (95% CI)	OR[c] (95% CI)
LnBPb	4.388 (2.223, 8.661) ***	4.773 (2.334, 9.763) ***	3.543 (1.705, 7.363) **
Dopamine	1.168 (0.920, 1.482)	1.213 (0.951, 1.546)	1.080 (0.828, 1.409)
Substance P	2.755 (0.889, 8.535)	2.489 (0.788, 7.859)	1.131 (0.308, 4.151)
NPY	0.034 (0.003, 0.399) **	0.037 (0.003, 0.446) **	0.063 (0.004, 0.902) *

LnBPb, ln-transformed blood Pb level; OR, odds ratio; CI, confidence interval.
*p < 0.05. **p < 0.01. ***p < 0.001.
[a] Logistic regression model unadjusted.
[b] Adjusted for gender, age.
[c] Adjusted for gender, age, maternal education levels, monthly household income, left-behind child, mother's work involved in e-waste, and sucking/biting toys.

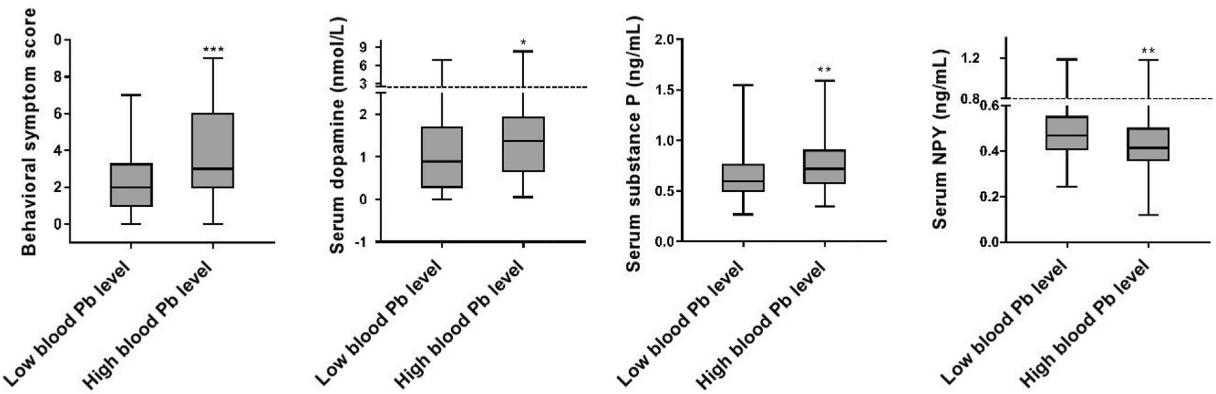

Fig. 4. Behavioral symptoms score, levels of serum dopamine and substance P from the high blood Pb level group were higher than from the low blood Pb level group. Serum NPY concentration from the low blood Pb level group were higher than from the high blood Pb level group. Data analysis by the Mann-Whitney *U* test. *$p < 0.05$. **$p < 0.01$. ***$p < 0.001$.

Based on the scores in the behavioral symptom subscale of the SDQ questionnaire, 48.2% of the children in the exposed group are categorized as at risk for behavioral difficulties, which is far higher than those with 13.9% in the reference group and other regions in the world. For instances, the prevalence rate of behavioral difficulties is 18.9% in children (3−17 years old) from Shanghai communities in eastern China, 13.7% in the Danish children (5−7 years old), 15.7% in Japanese children (4−12 years old), and 21.3% in German children (2−11 years old) (Du et al., 2008; Matsuishi et al., 2008; Elberling et al., 2010; Herrmann et al., 2018). All the above-mentioned studies used the parent report version of the SDQ, as well as the same bandings and cut-offs. Analysis of relevant factors indicates that behavioral symptom scores positively correlate with blood Pb level and number of e-waste sites within 50 m of dwelling. Logistic regression analysis suggest that Pb exposure is a risk factor exerting behavioral difficulties in children. Additionally, higher levels of maternal education and monthly household income are related to lower child's behavioral difficulties, which is in accordance with the previous findings (Bongers et al., 2003; Holling et al., 2008; Herrmann et al., 2018). There is an explanation that children whose parents are highly educated and have high incomes may pay more attention to the development of their children's behavior and intelligence. On the other hand, children whose parents have not received higher education or earn less incomes may receive less attention in terms of behavior, which, in turn, can have significant impact on their behavior. We also found that, the prevalence of behavioral difficulties in girls is higher than in boys, which is supported by a previous study suggesting that gender differences might exist in behavioral difficulties (Holling et al., 2008). Gender differences in behavioral difficulties may be caused by multi-factor integration, such as physiological factors (including estrogen fluctuations, HPA axis imbalance, and GABA disorder), and genetic susceptibility factors, as well as the parenting style (Li et al., 2015). Furthermore, we found that the left-behind children suffer from higher rate of behavioral difficulties, which has proven to be a risk factor of child's behavioral health (Sun et al., 2017).

Previous studies have indicated that Pb can induce oxidative stress, change Ca-dependent reaction, and unsettle BBB (Nava-Ruiz et al., 2012; Wu et al., 2020). Pb exposure and subsequently impairment of BBB in structure and function affect the interplay and transport of neurotransmitters between circulation and brain. In order to further estimate the influence of Pb exposure derived from e-waste dismantling activities on the behavioral health status in children, we classified all participants as having high blood Pb levels (BPb ≥ 5 μg/dL) and low blood Pb levels (BPb < 5 μg/dL), then compared scores and biomarker levels. After categorizing the children into high and low groups by their blood Pb, the behavioral symptom scores is significantly higher in the blood Pb levels ≥ 5 μg/dL group than those in the blood Pb levels <5 μg/dL group, which may suggest that Pb exposure may increase the risk for children's behavioral difficulties. In addition, the median of substance P and dopamine in children from the high blood Pb level group was significantly higher than those from the low blood Pb level group. Conversely, the level of NPY in children of the low blood Pb level group was higher than the children in the high blood Pb level group. It is well known that emotion-stimuli induces presynaptic glutamate release at the initial stage of behavioral processing, which takes a key role in inducing of synaptic plasticity by Ca^{2+} dependent pathway and postsynaptic NMDA receptor activation (Abe, 2001; Barkus et al., 2010; Marsden, 2013; Duman et al., 2016). In addition, there is evidence that Pb restrains postsynaptic NMDA receptors, and thus presynaptic glutamate release usually cannot activate postsynaptic NMDA receptor (Sanders et al., 2009; Baranowska-Bosiacka et al., 2012; Liu et al., 2013).

One of our previous studies, in regard to the effects of Pb on NMDA receptors, showed that a compensatory increase of presynaptic BDNF release led to more glutamate release in Pb-exposed children (Zhang et al., 2017). Due to the antagonistic effect between glutamate and NPY, more glutamate release inhibits the expression of NPY in the limbic system of Pb-exposed children, which could be demonstrated by the negative correlation between serum NPY and blood Pb level in our present study (Silva et al., 2005). Together, the Spearman correlation and binary logistic regression analysis findings indicate that serum NPY is the most important biomarker contributing to the risk of behavioral difficulties, which is consistent with several animal studies (Redrobe et al., 2002; Gelfo et al., 2012). It is noticeable that NPY acts a crucial signaling part in anti-anxiety and anti-depression through Y1 receptors. As well as NPY, the substance P/NK1 receptor activation may phasically participate in several specific aspects of behavioral problems (Mantyh, 2002). Our study shows that children with a higher substance P levels will be more likely to have behavioral difficulties, which is consistent with the results of previous studies (Kramer et al., 1998; Herpfer et al., 2005; Katsouni, 2009; Bassi et al., 2014). Additionally, there was no significant association observed between dopamine with the risk of behavioral difficulties in the logistic regression analysis, suggesting that dopamine might not be a biomarker of child's behavioral difficulties. From the above findings, we conclude that Pb undermines the NMDA receptors in emotion-related brain areas, for instance, the hippocampus, amygdaloid nucleus, cingulate cortex, and hypothalamus, causing unnatural upstream and downstream molecular events, such as

NPY-Y1, substance P-NK1 and Ca^{2+} signaling pathways, resulting in behavioral difficulties.

Epidemiological associations between childhood Pb exposure, as a purported risk factor for delinquent behavior, and criminal arrests in early adulthood have been investigated by previous studies (Fergusson et al., 2008; Wright et al., 2008; Haynes et al., 2011; Beckley et al., 2018). The present study is the first to observe an association between blood Pb level and fundamental neuropsychological mechanisms (e.g. impaired emotion regulation and increased hostile distrust) of preschool children living in an e-waste dismantling area that might explain these above associations with more complex outcomes. Childhood Pb exposure has been shown to generate neural dysfunction in areas of the brain involved in emotion, behavioral inhibition, and judgment (Lidsky and Schneider, 2003; Cecil et al., 2008; Brubaker et al., 2010; Beckwith et al., 2018). This could result in poor impulse control and underlying neurobehavioral deficits, which related with a higher probability of criminal behavior at older ages (Bellinger, 2008).

Our current study has some limitations. Firstly, we use a parent-reported measurement to assess behavioral difficulties in children, which may be biased as compared with the actual behavioral health status. However, the validity and reliability of the SDQ have been well documented in many studies. Secondly, although this cross-sectional study provides a correlation between Pb and biochemical indicators, which does not prove the causal relationship. Thirdly, the underlying mechanism of Pb on the neurotransmitter and behavioral alteration is still need further investigated and confirmed. Last but not least, we did not investigate the effects of the other contaminants due to insufficient blood sample. Therefore, future studies should focus on the joint toxicity of multiple pollutants on behavioral health.

5. Conclusion

In summary, the present research finds that the prevalence of behavioral difficulties in children from Guiyu is higher (48.2%) than those from the reference area. The results of this study show that Pb exposure in e-waste recycling regions lead to a decrease in serum NPY levels, an increase in substance P concentration, and a higher risk for behavioral difficulties. Additionally, NPY and substance P might be associated with the behavioral health of children. Taken together, strategies to decrease the Pb exposure will contribute to improve the mental and behavioral health of adulthood and future generations.

Credit author statement

Xiang Zeng: Writing - original draft, Conceptualization. Cheng Xu: Writing - original draft. Xijin Xu: Conceptualization, Supervision, Writing - review & editing, Validation. Yu Zhang: Writing - review & editing. Yu Huang: Writing - review & editing. Xia Huo: Conceptualization, Supervision, Writing - review & editing, Validation, Funding acquisition.

Compliance with ethical standards

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments. This study protocol was approved by the Human Ethics Committee of Shantou University Medical College, China. All parents and guardians of children agreed to enroll, and offered written informed-consent for participation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Abe, K., 2001. Modulation of hippocampal long-term potentiation by the amygdala: a synaptic mechanism linking emotion and memory. Jpn. J. Pharmacol. 86, 18–22.
Alassali, A., Barouta, D., Tirion, H., Moldt, Y., Kuchta, K., 2020. Towards a high quality recycling of plastics from waste electrical and electronic equipment through separation of contaminated fractions. J. Hazard Mater. 387, 121741.
Badgaiyan, R.D., 2010. Dopamine is released in the striatum during human emotional processing. Neuroreport 21, 1172–1176.
Baranowska-Bosiacka, I., Gutowska, I., Rybicka, M., Nowacki, P., Chlubek, D., 2012. Neurotoxicity of lead. Hypothetical molecular mechanisms of synaptic function disorders. Neurol. Neurochir. Pol. 46, 569–578.
Barkus, C., McHugh, S.B., Sprengel, R., Seeburg, P.H., Rawlins, J.N., Bannerman, D.M., 2010. Hippocampal NMDA receptors and anxiety: at the interface between cognition and emotion. Eur. J. Pharmacol. 626, 49–56.
Bassi, G.S., de Carvalho, M.C., Brandao, M.L., 2014. Effects of substance P and Sar-Met-SP, a NK1 agonist, in distinct amygdaloid nuclei on anxiety-like behavior in rats. Neurosci. Lett. 569, 121–125.
Beckley, A.L., Caspi, A., Broadbent, J., Harrington, H., Houts, R.M., Poulton, R., Ramrakha, S., Reuben, A., Moffitt, T.E., 2018. Association of childhood blood lead levels with criminal offending. JAMA Pediatr 172, 166–173.
Beckwith, T.J., Dietrich, K.N., Wright, J.P., Altaye, M., Cecil, K.M., 2018. Reduced regional volumes associated with total psychopathy scores in an adult population with childhood lead exposure. Neurotoxicology 67, 1–26.
Bellinger, D.C., 2008. Neurological and behavioral consequences of childhood lead exposure. PLoS Med. 5, e115.
Betts, Kellyn S, 2012. CDC updates guidelines for children's lead exposure. Environ. Health Perspect. 120 (7) https://doi.org/10.1289/ehp.120-a268 Free PMC article a268-a268.
Bongers, I.L., Koot, H.M., van der Ende, J., Verhulst, F.C., 2003. The normative development of child and adolescent problem behavior. J. Abnorm. Psychol. 112, 179–192.
Bouyatas, M.M., Abbaoui, A., Gamrani, H., 2019. Neurobehavioral effects of acute and chronic lead exposure in a desert rodent Meriones shawi: involvement of serotonin and dopamine. J. Chem. Neuroanat. 102, 101689.
Brubaker, C.J., Dietrich, K.N., Lanphear, B.P., Cecil, K.M., 2010. The influence of age of lead exposure on adult gray matter volume. Neurotoxicology 31, 259–266.
Canfield, R.L., Henderson, C.J., Cory-Slechta, D.A., Cox, C., Jusko, T.A., Lanphear, B.P., 2003. Intellectual impairment in children with blood lead concentrations below 10 microg per deciliter. N. Engl. J. Med. 348, 1517–1526.
Cecil, K.M., Brubaker, C.J., Adler, C.M., Dietrich, K.N., Altaye, M., Egelhoff, J.C., Wessel, S., Elangovan, I., Hornung, R., Jarvis, K., Lanphear, B.P., 2008. Decreased brain volume in adults with childhood lead exposure. PLoS Med. 5, e112.
Chang, J., Kueon, C., Kim, J., 2014. Influence of lead on repetitive behavior and dopamine metabolism in a mouse model of iron overload. Toxicol Res 30 (4), 267–276.
Chang, S., Fan, Y., Shin, J.H., Ryu, Y., Kim, M.S., Steffensen, S.C., Kim, H.K., Kim, J.M., Lee, B.H., Jang, E.Y., Yang, C.H., Kim, H.Y., 2019. Unpleasant sound elicits negative emotion and reinstates drug seeking. Mol. Neurobiol. 56, 7594–7607.
Chen, Y., Xu, X., Zeng, Z., Lin, X., Qin, Q., Huo, X., 2019. Blood lead and cadmium levels associated with hematological and hepatic functions in patients from an e-waste-polluted area. Chemosphere 220, 531–538.
Chen, Z., Huo, X., Zhang, S., Cheng, Z., Huang, Y., Xu, X., 2020. Relations of blood lead levels to echocardiographic left ventricular structure and function in preschool children. Chemosphere 28, 128793.
Dalgleish, T., 2004. The emotional brain. Nat. Rev. Neurosci. 5, 583–589.
Datar, P., Srivastava, S., Coutinho, E., Govil, G., 2004. Substance P: structure, function, and therapeutics. Curr. Top. Med. Chem. 4, 75–103.
de Souza, L.S., Goncalves, G., Komatsu, F., Queiroz, C.A., Almeida, A.A., Moreira, E.G., 2005. Developmental lead exposure induces depressive-like behavior in female rats. Drug Chem. Toxicol. 28, 67–77.
Du, Y., Kou, J., Coghill, D., 2008. The validity, reliability and normative scores of the parent, teacher and self report versions of the Strengths and Difficulties Questionnaire in China. Child Adolesc. Psychiatr. Ment. Health 2, 8.
Duman, R.S., Aghajanian, G.K., Sanacora, G., Krystal, J.H., 2016. Synaptic plasticity

and depression: new insights from stress and rapid-acting antidepressants. Nat. Med. 22, 238–249.
Ebner, K., Singewald, N., 2006. The role of substance P in stress and anxiety responses. Amino Acids 31, 251–272.
Elberling, H., Linneberg, A., Olsen, E.M., Goodman, R., Skovgaard, A.M., 2010. The prevalence of SDQ-measured mental health problems at age 5-7 years and identification of predictors from birth to preschool age in a Danish birth cohort: the Copenhagen Child Cohort 2000. Eur. Child Adolesc. Psychiatr. 19, 725–735.
Enman, N.M., Sabban, E.L., McGonigle, P., Van Bockstaele, E.J., 2015. Targeting the neuropeptide Y system in stress-related psychiatric disorders. Neurobiol Stress 1, 33–43.
Fergusson, D.M., Boden, J.M., Horwood, L.J., 2008. Dentine lead levels in childhood and criminal behaviour in late adolescence and early adulthood. J. Epidemiol. Community Health 62, 1045–1050.
Freire, C., Amaya, E., Gil, F., Fernandez, M.F., Murcia, M., Llop, S., Andiarena, A., Aurrekoetxea, J., Bustamante, M., Guxens, M., Ezama, E., Fernandez-Tardon, G., Olea, N., 2018. Prenatal co-exposure to neurotoxic metals and neurodevelopment in preschool children: the Environment and Childhood (INMA) Project. Sci. Total Environ. 621, 340–351.
Friedman, A., Kaufer, D., 2015. Blood-brain barrier in health and disease. Semin. Cell Dev. Biol. 38, 1.
Garza, A., Vega, R., Soto, E., 2006. Cellular mechanisms of lead neurotoxicity. Med. Sci. Mon. Int. Med. J. Exp. Clin. Res. 12 (3), 57–65.
Gelfo, F., Tirassa, P., De Bartolo, P., Croce, N., Bernardini, S., Caltagirone, C., Petrosini, L., Angelucci, F., 2012. NPY intraperitoneal injections produce antidepressant-like effects and downregulate BDNF in the rat hypothalamus. CNS Neurosci. Ther. 18, 487–492.
Goodman, R., Ford, T., Simmons, H., Gatward, R., Meltzer, H., 2003. Using the Strengths and Difficulties Questionnaire (SDQ) to screen for child psychiatric disorders in a community sample. Int. Rev. Psychiatr. 15, 166–172.
Gump, B.B., Dykas, M.J., MacKenzie, J.A., Dumas, A.K., Hruska, B., Ewart, C.K., Parsons, P.J., Palmer, C.D., Bendinskas, K., 2017. Background lead and mercury exposures: psychological and behavioral problems in children. Environ. Res. 158, 576–582.
Hansel, D.E., Eipper, B.A., Ronnett, G.V., 2001. Neuropeptide Y functions as a neuroproliferative factor. Nature 410 (6831), 940–944.
Haynes, E.N., Chen, A., Ryan, P., Succop, P., Wright, J., Dietrich, K.N., 2011. Exposure to airborne metals and particulate matter and risk for youth adjudicated for criminal activity. Environ. Res. 111, 1243–1248.
Heacock, M., Kelly, C.B., Asante, K.A., Birnbaum, L.S., Bergman, A.L., Brune, M.N., Buka, I., Carpenter, D.O., Chen, A., Huo, X., Kamel, M., Landrigan, P.J., Magalini, F., Diaz-Barriga, F., Neira, M., Omar, M., Pascale, A., Ruchirawat, M., Sly, L., Sly, P.D., Van den Berg, M., Suk, W.A., 2016. E-Waste and harm to vulnerable populations: a growing global problem. Environ. Health Perspect. 124, 550–555.
Herpfer, I., Lieb, K., 2005. Substance P receptor antagonists in psychiatry: rationale for development and therapeutic potential. CNS Drugs 19 (4), 275–293.
Heilig, M., 2004. The NPY system in stress, anxiety and depression. Neuropeptides 38, 213–224.
Heilig, M., Thorsell, A., 2002. Brain neuropeptide Y (NPY) in stress and alcohol dependence. Rev. Neurosci. 13, 85–94.
Herpfer, I., Katzev, M., Feige, B., Fiebich, B.L., Voderholzer, U., Lieb, K., 2007. Effects of substance P on memory and mood in healthy male subjects. Hum. Psychopharmacol. 22, 567–573.
Herrmann, J., Vogel, M., Pietzner, D., Kroll, E., Wagner, O., Schwarz, S., Muller, E., Kiess, W., Richter, M., Poulain, T., 2018. Factors associated with the emotional health of children: high family income as a protective factor. Eur. Child Adolesc. Psychiatr. 27, 319–328.
Holling, H., Kurth, B.M., Rothenberger, A., Becker, A., Schlack, R., 2008. Assessing psychopathological problems of children and adolescents from 3 to 17 years in a nationwide representative sample: results of the German health interview and examination survey for children and adolescents (KiGGS). Eur. Child Adolesc. Psychiatr. 17 (Suppl. 1), 34–41.
Horton, C.J., Acharya, L., Wells, E.M., 2019. Association between self-reported length of time in the USA and blood lead levels: national Health and Nutrition Examination Survey 2013-2016. BMJ Open 9, e27628.
Hou, R., Huo, X., Zhang, S., Xu, C., Huang, Y., Xu, X., 2020. Elevated levels of lead exposure and impact on the anti-inflammatory ability of oral sialic acids among preschool children in e-waste areas. Sci. Total Environ. 699, 134380.
Huang, P.C., Su, P.H., Chen, H.Y., Huang, H.B., Tsai, J.L., Huang, H.I., Wang, S.L., 2012. Childhood blood lead levels and intellectual development after ban of leaded gasoline in Taiwan: a 9-year prospective study. Environ. Int. 40, 88–96.
Huo, X., Peng, L., Xu, X., Zheng, L., Qiu, B., Qi, Z., Zhang, B., Han, D., Piao, Z., 2007. Elevated blood lead levels of children in Guiyu, an electronic waste recycling town in China. Environ. Health Perspect. 115, 1113–1117.
Iftikhar, K., Siddiq, A., Baig, S.G., Zehra, S., 2020. Substance P: a neuropeptide involved in the psychopathology of anxiety disorders. Neuropeptides 79, 101993.
Israelov, H., Ravid, O., Atrakchi, D., Rand, D., Elhaik, S., Bresler, Y., et al., 2020. Caspase-1 has a critical role in blood-brain barrier injury and its inhibition contributes to multifaceted repair. J. Neuroinflammation 17 (1), 267.
Jansen, P.W., Duijff, S.N., Beemer, F.A., Vorstman, J.A., Klaassen, P.W., Morcus, M.E., Heineman-de, B.J., 2007. Behavioral problems in relation to intelligence in children with 22q11.2 deletion syndrome: a matched control study. Am. J. Med. Genet. 143A, 574–580.
Katsouni, E., Sakkas, P., Zarros, A., Skandali, N., Liapi, C., et al., 2009. The involvement of substance P in the induction of aggressive behavior. Peptides 30 (8), 1586–1591.
Koller, K., Brown, T., Spurgeon, A., Levy, L., 2004. Recent developments in low-level lead exposure and intellectual impairment in children. Environ. Health Perspect. 112, 987–994.
Kordas, K., Burganowski, R., Roy, A., Peregalli, F., Baccino, V., Barcia, E., Mangieri, S., Ocampo, V., Manay, N., Martinez, G., Vahter, M., Queirolo, E.I., 2018. Nutritional status and diet as predictors of children's lead concentrations in blood and urine. Environ. Int. 111, 43–51.
Kramer, M.S., Cutler, N., Feighner, J., Shrivastava, R., Carman, J., Sramek, J.J., et al., 1998. Distinct mechanism for antidepressant activity by blockade of central substance P receptors. Science 281 (5383), 1640–1645.
Leung, A.O., Duzgoren-Aydin, N.S., Cheung, K.C., Wong, M.H., 2008. Heavy metals concentrations of surface dust from e-waste recycling and its human health implications in southeast China. Environ. Sci. Technol. 42, 2674–2680.
Li, M., Lu, S., Wang, G., Zhong, N., 2015. The effects of gender differences in patients with depression on their emotional working memory and emotional experience. Behav. Neurol. 2015, 807343.
Lidsky, T.I., Schneider, J.S., 2003. Lead neurotoxicity in children: basic mechanisms and clinical correlates. Brain 126, 5–19.
Liu, J., Liu, X., Wang, W., McCauley, L., Pinto-Martin, J., Wang, Y., Li, L., Yan, C., Rogan, W.J., 2014a. Blood lead concentrations and children's behavioral and emotional problems: a cohort study. JAMA Pediatr 168, 737–745.
Liu, J., Xu, X., Wu, K., Piao, Z., Huang, J., Guo, Y., Li, W., Zhang, Y., Chen, A., Huo, X., 2011. Association between lead exposure from electronic waste recycling and child temperament alterations. Neurotoxicology 32, 458–464.
Liu, K.S., Hao, J.H., Zeng, Y., Dai, F.C., Gu, P.Q., 2013. Neurotoxicity and biomarkers of lead exposure: a review. Chin. Med. Sci. J. 28, 178–188.
Liu, L., Xu, X., Yekeen, T.A., Lin, K., Li, W., Huo, X., 2015. Assessment of association between the dopamine D2 receptor (DRD2) polymorphism and neurodevelopment of children exposed to lead. Environ. Sci. Pollut. Res. Int. 22 (3), 1786–1793.
Liu, L., Zhang, B., Lin, K., Zhang, Y., Xu, X., Huo, X., 2018. Thyroid disruption and reduced mental development in children from an informal e-waste recycling area: a mediation analysis. Chemosphere 193, 498–505.
Liu, W., Huo, X., Liu, D., Zeng, X., Zhang, Y., Xu, X., 2014b. S100β in heavy metal-related child attention-deficit hyperactivity disorder in an informal e-waste recycling area. Neurotoxicology 45, 185–191.
Lorente, L., Martin, M.M., Perez-Cejas, A., Gonzalez-Rivero, A.F., Argueso, M., Ramos, L., Sole-Violan, J., Caceres, J.J., Jimenez, A., Garcia-Marin, V., 2019. Persistently high serum substance P levels and early mortality in patients with severe traumatic brain injury. World Neurosurg 132, e613–e617.
Mantyh, P.W., 2002. Neurobiology of substance P and the NK1 receptor. J. Clin. Psychiatr. 63 (Suppl. 11), 6–10.
Marsden, W.N., 2013. Synaptic plasticity in depression: molecular, cellular and functional correlates. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 43, 168–184.
Matsuishi, T., Nagano, M., Araki, Y., Tanaka, Y., Iwasaki, M., Yamashita, Y., Nagamitsu, S., Iizuka, C., Ohya, T., Shibuya, K., Hara, M., Matsuda, K., Tsuda, A., Kakuma, T., 2008. Scale properties of the Japanese version of the Strengths and Difficulties Questionnaire (SDQ): a study of infant and school children in community samples. Brain Dev. 30, 410–415.
Mielke, H.W., Gonzales, C.R., Powell, E.T., Shah, A., Berry, K.J., Richter, D.D., 2020. Spatial-temporal association of soil Pb and children's blood Pb in the Detroit Tri-County Area of Michigan (USA). Environ. Res. 191, 110112.
Moreira, E.G., Vassilieff, I., Vassilieff, V.S., 2001. Developmental lead exposure: behavioral alterations in the short and long term. Neurotoxicol. Teratol. 23 (5), 489–495.
Morales-Medina, J.C., Dumont, Y., Quirion, R., 2010. A possible role of neuropeptide Y in depression and stress. Brain Res. 1314, 194–205.
Nation, D.A., Sweeney, M.D., Montagne, A., Sagare, A.P., D'Orazio, L.M., Pachicano, M., 2019. Blood-brain barrier breakdown is an early biomarker of human cognitive dysfunction. Nat. Med. 25 (2), 270–276.
Nava-Ruiz, C., Méndez-Armenta, M., Ríos, C., 2012. Lead neurotoxicity: effects on brain nitric oxide synthase. J. Mol. Histol. 43 (5), 553–563.
Nehru, B., Sidhu, P., 2001. Behavior and neurotoxic consequences of lead on rat brain followed by recovery. Biol. Trace Elem. Res. 84 (1–3), 113–121.
Nieoullon, A., Coquerel, A., 2003. Dopamine: a key regulator to adapt action, emotion, motivation and cognition. Curr. Opin. Neurol. 16 (Suppl. 2), S3–S9.
Philippat, C., Nakiwala, D., Calafat, A.M., Botton, J., De Agostini, M., Heude, B., Slama, R., 2017. Prenatal exposure to nonpersistent endocrine disruptors and behavior in boys at 3 and 5 years. Environ. Health Perspect. 125, 97014.
Priyadharsini, T., Singh, M.E.M., 2019. E-waste and health effects, an Emerging issue. Asian Journal of Nursing Education and Research 9, 570–572.
Profaci, C.P., Munji, R.N., Pulido, R.S., Daneman, R., 2020. The blood-brain barrier in health and disease: important unanswered questions. J. Exp. Med. 217 (4), e20190062.
Redrobe, J.P., Dumont, Y., Fournier, A., Quirion, R., 2002. The neuropeptide Y (NPY) Y1 receptor subtype mediates NPY-induced antidepressant-like activity in the mouse forced swimming test. Neuropsychopharmacology 26, 615–624.
Rice, D.C., 1996. Behavioral effects of lead: commonalities between experimental and epidemiologic data. Environ. Health Perspect. 104 (Suppl. 2), 337–351. Suppl 2.
Robinson, B.H., 2009. E-waste: an assessment of global production and environmental impacts. Sci. Total Environ. 408, 183–191.

Sah, R., Geracioti, T.D., 2013. Neuropeptide Y and posttraumatic stress disorder. Mol. Psychiatr. 18, 646–655.

Sanchez-de-la-Torre, M., Barcelo, A., Pierola, J., Esquinas, C., de la Pena, M., Duran-Cantolla, J., Capote, F., Masa, J.F., Marin, J.M., Vila, M., Cao, G., Martinez, M., de Lecea, L., Gozal, D., Montserrat, J.M., Barbe, F., 2011. Plasma levels of neuropeptides and metabolic hormones, and sleepiness in obstructive sleep apnea. Respir. Med. 105, 1954–1960.

Sanders, T., Liu, Y., Buchner, V., Tchounwou, P.B., 2009. Neurotoxic effects and biomarkers of lead exposure: a review. Rev. Environ. Health 24, 15–45.

Sciarillo, W.G., Alexander, G., Farrell, K.P., 1992. Lead exposure and child behavior. Am. J. Publ. Health 82, 1356–1360.

Silva, A.P., Xapelli, S., Grouzmann, E., Cavadas, C., 2005. The putative neuroprotective role of neuropeptide Y in the central nervous system. Curr. Drug Targets - CNS Neurol. Disord. 4, 331–347.

Sioen, I., Den Hond, E., Nelen, V., Van de Mieroop, E., Croes, K., Van Larebeke, N., Nawrot, T.S., Schoeters, G., 2013. Prenatal exposure to environmental contaminants and behavioural problems at age 7-8years. Environ. Int. 59, 225–231.

Song, Q., Li, J., 2014. A systematic review of the human body burden of e-waste exposure in China. Environ. Int. 68, 82–93.

Strafella, A.P., 2019. Mesolimbic dopamine and anterior cingulate cortex connectivity changes lead to impulsive behaviour in Parkinson's disease. Brain 142 (3), 496–498.

Sun, M., Xue, Z., Zhang, W., Guo, R., Hu, A., Li, Y., Mwansisya, T.E., Zhou, L., Liu, C., Chen, X., Huang, X., Tao, H., Shi, J., Liu, Z., Rosenheck, R., 2017. Psychotic-like experiences, trauma and related risk factors among "left-behind" children in China. Schizophr. Res. 181, 43–48.

Taylor, C.M., Kordas, K., Golding, J., Emond, A.M., 2017. Data relating to prenatal lead exposure and child IQ at 4 and 8 years old in the Avon Longitudinal Study of Parents and Children. Neurotoxicology 62, 224–230.

Wong, C.S., Duzgoren-Aydin, N.S., Aydin, A., Wong, M.H., 2007. Evidence of excessive releases of metals from primitive e-waste processing in Guiyu, China. Environ. Pollut. 148 (1), 62–72.

Wright, J.P., Dietrich, K.N., Ris, M.D., Hornung, R.W., Wessel, S.D., Lanphear, B.P., Ho, M., Rae, M.N., 2008. Association of prenatal and childhood blood lead concentrations with criminal arrests in early adulthood. PLoS Med. 5, e101.

Wu, K., Xu, X., Liu, J., Guo, Y., Li, Y., Huo, X., 2010. Polybrominated diphenyl ethers in umbilical cord blood and relevant factors in neonates from Guiyu, China. Environ. Sci. Technol. 44, 813–819.

Wu, S., Liu, H., Zhao, H., Wang, X., Chen, J., Xia, D., 2020. Environmental lead exposure aggravates the progression of Alzheimer's disease in mice by targeting on blood brain barrier. Toxicol. Lett. 319, 138–147.

Xu, X., Liu, J., Huang, C., Lu, F., Chiung, Y.M., Huo, X., 2015. Association of polycyclic aromatic hydrocarbons (PAHs) and lead co-exposure with child physical growth and development in an e-waste recycling town. Chemosphere 139, 295–302.

Zenaro, E., Piacentino, G., Constantin, G., 2017. The blood-brain barrier in Alzheimer's disease. Neurobiol. Dis. 107, 41–56.

Zeng, X., Huo, X., Xu, X., Liu, D., Wu, W., 2020. E-waste lead exposure and children's health in China. Sci. Total Environ. 734, 139286.

Zeng, X., Xu, X., Boezen, H.M., Huo, X., 2016a. Children with health impairments by heavy metals in an e-waste recycling area. Chemosphere 148, 408–415.

Zeng, X., Xu, X., Boezen, H.M., Vonk, J.M., Wu, W., Huo, X., 2017a. Decreased lung function with mediation of blood parameters linked to e-waste lead and cadmium exposure in preschool children. Environ. Pollut. 230, 838–848.

Zeng, X., Xu, X., Qin, Q., Ye, K., Wu, W., Huo, X., 2019. Heavy metal exposure has adverse effects on the growth and development of preschool children. Environ. Geochem. Health 41, 309–321.

Zeng, X., Xu, X., Zhang, Y., Li, W., Huo, X., 2017b. Chest circumference and birth weight are good predictors of lung function in preschool children from an e-waste recycling area. Environ. Sci. Pollut. Res. 24, 22613–22621.

Zeng, X., Xu, X., Zheng, X., Reponen, T., Chen, A., Huo, X., 2016b. Heavy metals in $PM_{2.5}$ and in blood, and children's respiratory symptoms and asthma from an e-waste recycling area. Environ. Pollut. 210, 346–353.

Zhang, B., Huo, X., Xu, L., Cheng, Z., Cong, X., Lu, X., Xu, X., 2017. Elevated lead levels from e-waste exposure are linked to decreased olfactory memory in children. Environ. Pollut. 231, 1112–1121.

Zhang, K., Schnoor, J.L., Zeng, E.Y., 2012. E-waste recycling: where does it go from here? Environ. Sci. Technol. 46, 10861–10867.

Zhang, Y., Huo, X., Lu, X., Zeng, Z., Faas, M.M., Xu, X., 2020. Exposure to multiple heavy metals associate with aberrant immune homeostasis and inflammatory activation in preschool children. Chemosphere 127257.

Zhao, T.T., Chen, B., Wang, H.P., Wang, R., Zhang, H., 2013. Evaluation of toxic and essential elements in whole blood from 0- to 6-year-old children from Jinan, China. Clin. Biochem. 46, 612–616.

Zheng, X., Huo, X., Zhang, Y., Wang, Q., Zhang, Y., Xijin, X., 2019. Cardiovascular endothelial inflammation by chronic coexposure to lead (Pb) and polycyclic aromatic hydrocarbons from preschool children in an e-waste recycling area. Environ. Pollut. 246, 587–596.