NeuroToxicology 62 (2017) 186–191

Contents lists available at ScienceDirect

NeuroToxicology

Full Length Article

Strain specific effects of low level lead exposure on associative learning and memory in rats

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ARTICLE INFO

Article history:
Received 31 May 2017
Received in revised form 1 July 2017
Accepted 12 July 2017
Available online 16 July 2017

Keywords:
Developmental lead exposure
Trace fear conditioning
Strain differences

ABSTRACT

Exposure to lead (Pb) remains a significant public health concern. Lead exposure in early life impairs the normal development of numerous cognitive and neurobehavioral processes. Previous work has shown that the effects of developmental Pb exposure on gene expression patterns in the brain are modulated by various factors including the developmental timing of the exposure, level of exposure, sex, and genetic background. Using gene microarray profiling, we previously reported a significant strain-specific effect of Pb exposure on the hippocampal transcriptome, with the greatest number of differentially expressed transcripts in Long Evans (LE) rats and the fewest in Sprague Dawley (SD) rats. The present study examined the extent to which this differential effect of Pb on hippocampal gene expression might influence behavior. Animals (males and females) were tested in a trace fear conditioning paradigm to evaluate effects of Pb exposures (perinatal (PERI; gestation to postnatal day 21) or early postnatal (EPN; postnatal day 1 to day 21)) on associative learning and memory. All animals (Pb-exposed and non-Pb-exposed controls) showed normal acquisition of the conditioned stimulus (tone)-unconditioned stimulus (footshock) association. Long Evans rats showed a significant deficit in short- and long-term recall, influenced by sex and the timing of Pb exposure (PERI or EPN). In contrast, Pb exposure had no significant effect on memory consolidation or recall in any SD rats. These results further demonstrate the important influence of genetic background to the functional outcomes from developmental Pb exposure.

1. Introduction

Early life exposure to lead (Pb) impairs a variety of cognitive, behavioral and neurochemical processes resulting in a variety of potentially negative outcomes for children exposed to this potent neurotoxicant. Children with even low level Pb exposures can display cognitive/behavioral problems including deficits in learning, memory, attention, and executive function, as well as increased impulsivity and aggression (Cecil et al., 2008; Cecil and Kos, 2006; Mazumdar et al., 2011; Nigg et al., 2010; Surkan et al., 2007). Yet, developmental Pb exposure does not result in the same array or extent of deficits in all exposed individuals and consequently, no specific Pb-associated "behavioral signature" has been identified. Individuals with similar Pb exposures can express different behavioral and cognitive impairments and express different profiles of neuropsychological impairments even when subjects are assessed using the same battery of tests (Lidsky and Schneider, 2006). While a number of factors including home environment, socioeconomic status, and race can influence the susceptibility for Pb exposure (ex., (Chung et al., 2001; Gellert et al., 1993), genetic factors may also affect the toxicokinetics of Pb and the brain's vulnerability to its neurotoxic effects (Onalaja and Claudio, 2000; Stewart et al., 2002). Additionally, genetic factors may also influence the functional outcomes from developmental Pb exposure.

While genomic variation can influence the phenotypic expression of a variety of traits, genomic variation may also influence the manner in which the brain (or other organ systems) respond to a particular toxicant, stressor, or injury. In a recent study, we used gene profiling and microarray technology to identify potential strain differences in Pb-responsive genes in the hippocampus, a brain structure well known to be adversely affected by Pb, in rats of different genetic backgrounds (i.e., Fischer 344 (F344), Long Evans (LE), and Sprague Dawley (SD)). We found significant strain-related effects of Pb on the hippocampal transcriptome that were not due to strain-related differences in brain accumulation of Pb (Schneider et al., 2014). A large number of transcripts (978) were differentially expressed in LE rats across all experimental groups (male/female, perinatal or postnatal Pb exposure), while 269

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http://dx.doi.org/10.1016/j.neuro.2017.07.006
0161-813X/

transcripts were differentially expressed in F344 rats, and only 179 transcripts were differentially expressed in SD rats (Schneider et al., 2014). The results of this study demonstrated that at least at the level of gene transcription, the response of the brain to a given Pb exposure appears to be uniquely related to the strain of the animal and suggested the possibility that differences in neurobehavioral outcomes from developmental Pb exposure in different strains of animals could occur. As there is little known about the neurobehavioral influences of strain or genetic background on cognitive outcomes following developmental Pb exposure, the present study was conducted to investigate the role that genetic variation may play in influencing cognitive outcomes from developmental Pb exposure. As we have reported deficits in associative memory in Pb-exposed LE rats (the strain that we previously found to have the largest number of hippocampal transcripts altered by Pb exposure), the current study investigated the effects of similar Pb exposures (150 ppm) on associative learning/memory in SD rats (the strain that we previously found to have the fewest number of hippocampal transcripts differentially altered by Pb exposure), using a trace fear-conditioning paradigm.

2. Materials and methods

2.1. Animals and treatments

The use of animals was in compliance with NIH Guidelines for the Care and Use of Laboratory Animals and the study was approved by the institutional animal care and use committee at Thomas Jefferson University. Long Evans and Sprague Dawley rats and two Pb exposure paradigms (perinatal (PERI) exposure and early postnatal (EPN)) were used. The rats were acclimatized to the animal facility for at least a week before the start of the experiments. All experimental animals were generated in house to reduce outside sources of potential variability. In the PERI exposure group, 50–55 day old LE dams (Envigo Laboratories) and SD dams (Taconic Laboratories) were fed chow (RMH 1000) with or without added Pb acetate (150 ppm or 0 ppm) for 14 days prior to breeding and remained on the same diet through weaning, as previously described (Anderson et al., 2016). Thus, dams were continuously exposed to Pb for approximately 55–60 days. Litters were culled to equal numbers of pups to standardize litter size, with an aim of having eight pups per litter. Equal numbers of males and females were maintained wherever possible and were exposed to Pb from gestation through lactation (i.e., to postnatal day 21). At weaning, rats were housed four to a standard cage (940 cm^2) with *ad lib* access to chow (no added Pb) and water until behavioral testing, beginning at postnatal day 55. For animals in the EPN group, 50–55 day old dams were fed RMH 1000 chow with no added Pb during gestation and were then fed chow with or without added Pb acetate (150 ppm or 0 ppm) beginning at parturition and pups continued to receive the same exposure to Pb through weaning at postnatal day 21. All animals were exposed to a 12 h:12 h light:dark cycle for the duration of the experiment. The number of individuals (n) tested in each group were (LE and SD, respectively): Control (no Pb): male (n = 16, 18), female (n = 16, 18); Perinatal Pb exposure: male (n = 16, 18), female (n = 16, 18); Early postnatal Pb exposure: male (n = 16, 18), female (n = 16, 18). Throughout the study, all rats were weighed every two weeks to ensure that the Pb exposure was well tolerated. No more than one male and one female from any litter were included in any behavioral group to prevent litter effects.

2.2. Blood lead analysis

Trunk blood samples were collected into EDTA-containing collection tubes at time of euthanasia from littermates of experimental animals not used for behavioral studies on postnatal day 14 and from behaviorally tested animals at postnatal day 65 and analyzed for Pb levels using an ESA LeadCare II Blood Lead Analyzer system version 1.09 and LeadCare II Blood Lead Test kit (Magellan Diagnostics, MA). Briefly, 50 μl of whole blood was mixed with 250 μl of diluted hydrochloric acid solution (0.34 M) and was applied to the sensor strip of the LeadCare II Blood Lead Analyzer system. Values were recorded in μg/dL. Additionally, blood samples were obtained from dams by retro-orbital bleed before Pb exposure and at parturition and analyzed as described above.

2.3. Trace fear conditioning

Trace fear conditioning was carried out using Ugo Basile Fear Conditioning systems (30 cm deep x 34 cm wide x 41.5 cm high) and ANY-maze software (Version 4.99; Stoelting Co., Wood Dale, IL) that automatically measured the freezing response. The trace fear conditioning protocol consisted of habituation, acquisition training, and short- and long-term retrieval testing at Days 1, 2 and 10 post acquisition training as previously described (Anderson et al., 2016). Animals were habituated to the fear conditioning chamber, located within a dimly lit sound attenuating enclosure with white background noise, for 15 min one day prior to the acquisition trials. During acquisition trials, animals were given 180 s to habituate to the test chamber and then given a series of 6 pairings of tone (conditioned stimulus; CS, 3000 Hz, 80 dB for 15 s)-shock (unconditioned stimulus; US, 0.8 mA for 1.0 s) with a 20 s trace period between CS and US and pseudorandom inter-trial-intervals of 1–3 mins. Freezing behavior, defined by absence of all but respiratory movements, was measured every second during the 20 s of the trace period. Retention testing occurred at 1, 2 and 10 days post acquisition. For retention testing, animals were placed back into the same chamber in which they were initially trained but with different visual and olfactory cues. On each retention testing day, animals were habituated to the chamber for 180 s followed by presentation of 3 tones for 15 s each, in the absence of foot shock, with a pseudo random ITI (1–4 mins.) between presentation of tones. Freezing was measured every second during the 20 s after tone presentation.

2.4. Data analyses

Data were analyzed using a two-way analysis of variance (ANOVA) with repeated measure design followed by a Tukey test for post hoc analyses using GraphPad Prism (v. 7.02). Analysis of acquisition data included all six data points (trial 1–6) averaged to yield an estimate of percent time freezing. The analysis of the retention data (Days 1, 2 and 10) included only data from the first trial each day (Anderson et al., 2016). Statistical significance was defined at $p < 0.05$.

3. Results

3.1. Body weight analysis

Before the start of the Pb exposure, there were no within group differences in weights of males or females randomized to the Pb-exposure or the control group. There were no significant differences in body weights between controls and Pb-exposed groups within sex and strain throughout the study (data not shown). Additionally, there were no grossly observable differences in food or water consumption between the control and Pb-exposed LE and SD rats.