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# Epigenetic histone modification regulates developmental lead exposure induced hyperactivity in rats

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## HIGHLIGHTS

- Epigenetic changes in the histone modification level were proposed to be associated with lead induced hyperactivity.
- Hyperactivity caused by lead exposure was significantly influenced by doses.
- Dopaminergic proteins remained unchanged in the process of lead induced ADHD.
- Impairment caused by high-dose lead was hypothesized to be partly antagonized by histone acetylation.
- The increased expression of p300 was responsible for the hisone changes.

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## ABSTRACT

Lead (Pb) exposure was commonly considered as a high environmental risk factor for the development of attention-deficit/hyperactivity disorder (ADHD). However, the molecular basis of this pathological process still remains elusive. In light of the role of epigenetics in modulating the neurological disease and the causative environment, the alterations of histone modifications in the hippocampus of rats exposed by various doses of lead, along with concomitant behavioral deficits, were investigated in this study. According to the free and forced open field test, there showed that in a dosage-dependent manner, lead exposure could result in the increased locomotor activity of rats, that is, hyperactivity: a subtype of ADHD. Western blotting assays revealed that the levels of histone acetylation increased significantly in the hippocampus by chronic lead exposure, while no dramatic changes were detected in terms of expression yields of ADHD-related dopaminergic proteins, indicating that histone acetylation plays essential roles in this toxicant-involved pathogenesis. In addition, the increased level of histone acetylation might be attributed to the enzymatic activity of p300, a typical histone acetyltransferase, as the transcriptional level of p300 was significantly increased upon higher-dose Pb exposure. In summary, this study first discovered the epigenetic mechanism bridging the environmental influence (Pb) and the disease itself (ADHD) in the histone modification level, paving the way for the comprehensive understanding of ADHD's etiology and in further steps, establishing the therapy strategy of this widespread neurological disorder.


## 1. Introduction

Attention deficit hyperactivity disorder (ADHD) is one of the most common childhood neuropsychiatric disorders, occuring in an estimated 3% to 7% of school-aged children (Nigg et al., 2010). The etiology of ADHD was reported to be associated with both genetic and epigenetic factors, and a growing number of research suggested that exposure to environmental lead is a high risk factor for ADHD (Braun et al., 2006; Nigg et al., 2008; Wang et al., 2008). The heavy metal lead (Pb) is a well-studied toxicant known to cause the deficits of cognition (Cho et al., 2010; Ha et al., 2009), and has been proved to be associated with symptoms of inattention (Jakubowski, 2011).

There were a variety of causes including genetic factors, neurophysiological factors, mild brain injuries, psychological and social factors or multiple combinations of them leading to ADHD (Jones and Miller, 2008; Laucht et al., 2007). In addition, there was mounting evidence to suggest the pathophysiologically relevant contribution of monoaminergic pathways in the etiology of ADHD (Faraone and Mick, 2010; Peterson et al., 2011; Szczerbak et al., 2007; White et al., 2007; Zuch et al., 1998). It was demonstrated

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that dopamine transport gene (*DAT1*) and dopamine receptor gene (*DRD4*) were involved in the risk for ADHD (Hawi et al., 2005; Hoefgen et al., 2006; McCracken et al., 2000; Shumay et al., 2010).

The epigenetic factors, with particular relevance in psychopathology, could either strengthen or weaken the genetic effects through modifying the expression of individuals' genetic background on phenotypes (Archer et al., 2011; Rutten and Mill, 2009; Wilson, 2008). The post-translational modification of histones, the basic proteins around which DNA is wrapped to form nucleosomes, modulates gene expression via alterations in chromatin structure (Kaufmann et al., 2005; Pons et al., 2009; Timmermann et al., 2001). According to the limited epigenetic profiles reported by several studies on ADHD, histone modification patterns have the potential to become a useful marker to measure the disease (Egger et al., 2004; Seligson et al., 2005). But whether histone modification was involved in chronic lead-induced hyperactivity remains unknown.

Histone acetylation was considered to be the result of complex interaction between histone acetyltransferase (HAT) and histone deacetylase (HDAC) (Jakovcevski and Akbarian, 2012). HDAC1 was an important HDAC belonging to Class I and usually used as the model enzyme to investigate the function of HDAC inhibitors (Phiel et al., 2001). Besides, p300 was a critical trancription coactivator involved in extensive cellular processes and identified as a novel type of HAT (Ogryzko et al., 1996).

In this study, we performed a preliminary assessment of behavioral dysfunction and patterns of histone acetylation in lead-associated ADHD rats, and attempted to establish an exploratory correlation of these histone parameters with neurologic severity. The study could shed light on the epigenetic mechanisms lying behind the lead-associated ADHD.

## 2. Materials and methods

### 2.1. Experimental animals

Sprague-Dawley (SD) rats were supplied by the Laboratory Animal Center, Anhui Medical University, P.R. China, and were maintained in compliance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. The study was approved by the institutional animal care and use committee at Hefei University of Technology. Four female SD rats, weighing at 200–220 g, were randomly placed in four cages after mating. The rats were subsequently treated with distilled water containing a series of concentrations of lead (0 mg/L, 5 mg/L and 25 mg/L), respectively, till they gave birth to the next generation. Little pups in different groups were exposed to lead acetate during lactation indirectly through their mothers and then directly after weaning. Young rats were processed into different cages after weaning labeled according to sex and subsequently treated with different concentrations of lead acetate as mentioned above till 60 days old. Animals had free access to food and water. The room was maintained at 24–26 °C at 51–66% relative humidity on a 12-h alternating light/dark cycle.

### 2.2. Open-field test

To explore lead-induced behavior changes in rats, both the free-exploration open-field test (home cage accessible) and forced exploration open-field test (home cage not accessible) were performed as previously described (Sagvolden et al., 1993) with some modifications as follows:

#### 2.2.1. Free exploration open field

The experiment was conducted in the free exploration open-field apparatus. The open field measured $100 \times 100 \times 50$ (height) cm and was divided into $25 \times 20 \times 20$ squares by black paints. The 16 lattices near the wall were defined as the peripheral region and the remaining 9 lattices in the middle were defined as the intermediate region. There was a $41 \times 25$ cm hole in the center of the floor in which the rat's home cage can be placed during the test.

Each rat was carried into the open field for 30 min free moving to adapt to the environment during the last three days before testing. The rat was carried to the cage with minimal disturbance and the experimenter left the room quietly and started the session. Make records of each rat's activity within 5 min. The following measurements of behavior were recorded: number of intermediate regions covered with all four paws; latency to leave the home cage and enter the field; number of the peripheral regions covered with all four paws, as well as number of crossings from cage to field or vice versa. The rat's behavior was observed via a video camera which was installed above the open-field and connected to the computer. The videos were saved in the computer for later analysis.

#### 2.2.2. Forced exploration open field

This experiment was conducted using the same apparatus as the free exploration open field, except that the hole in the center of the floor was filled. The rat was removed from the home cage and placed in the middle lattice in the open field. The 5-min sessions were run and recorded. The following measures of behavior were recorded: number of intermediate regions covered with all four paws; number of the peripheral regions covered with all four paws.

### 2.3. Tissue preparation

On the last day of the open field test, animals were deeply anesthetized with $CO_2$ and blood was extracted from the abdominal veins. Then the rats were decapitated and the whole brain was collected. Half hippocampus was isolated for protein quantitative analysis, and the other half hippocampus was collected for lead concentration determination. The tissue samples from cerebral cortex were frozen at −80 °C and subsequently subject for the real time fluorescence quantitative PCR assay.

### 2.4. Lead concentration determination

Tissue Lead concentration assay was performed as follows: half hippocampus was added with nitric acid (excellent pure GR, 4 mL) and 30% hydrogen peroxide (AR, 2 mL) in the nitrolysis tube overnight at room temperature. And then, those tissues were nitrolyzed for 30 min in the microwave nitratepyrolysis furnace (EMR marsxpresscertificate, VB 20). Lastly, the lead concentration within sample was detected by the graphite furnace atomic spectrophotometry (the PerkinElmer AAnalyst$^{TM}$ 800, USA).

Blood lead concentration assay was performed as follows: 0.5 mL of blood was added into 4.5 mL dilution liquid (0.2% nitric acid and 0.1% TritonX-100). Each sample was vortexed for 2 min and the lead concentration within sample was detected by the graphite furnace atomic spectrophotometry (the PerkinElmer AAnalyst$^{TM}$ 800, USA).

### 2.5. Western blotting assay

The total protein levels were determined using the bicinchoninic acid (BCA) method. The proteins from the hippocampus were directly harvested by sample buffer solution. An equal amount of samples was resolved by an 8.5% SDS-PAGE gel. The resolved proteins were transferred to a PVDF membrane. The non-specific sites were blocked with 5% non-fat dry milk, followed by incubation with primary antibodies. The antibodies of β-actin, DAT1, DRD4, H3 and acetylated H3 were purchased from