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Chronic prenatal lead exposure impairs long-term memory in day old chicks

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ABSTRACT

Environmental exposure to lead during developmental stages has been established as a potential cause of intellectual deficits. The high susceptibility of rapidly developing fetal and infant brains to external factors suggests that impairment of later cognitive functions may arise from relatively minor prenatal exposure to environmental lead levels. In this study, we used the one-trial passive avoidance learning paradigm with day old chicks to evaluate memory function and memory consolidation in response to prenatal lead exposure. Lead acetate (5.5 mg/kg, 11 mg/kg, 16.5 mg/kg) was administered daily from E9 to E16 via direct injection into the airspace in chick eggs. Higher doses of lead acetate (11 mg/kg, 16.5 mg/kg) administration had significant effects on the hatching success (23.4 and 17, respectively) and hatch weight (~10% decrease) of chicks when compared to equivalent treatments of sodium acetate (11 mg/kg, 16.5 mg/kg) ($p < 0.001$). Low doses of lead acetate (5.5 mg/kg) did not significantly affect chick hatching, weight or morphology compared to equivalent sodium acetate treatments (5.5 mg/kg) and controls. However, lead acetate (5.5 mg/kg) was found to significantly impair long-term memory after 120 min following training in the one-trial passive avoidance learning task ($p < 0.05$). These findings add to a growing body of evidence that suggests lead toxicity during fetal development leads to impairment in cognitive and memory processes.

The human population is commonly exposed to trace amounts of lead present in air, soil or dust, drinking water and food, as well as in various consumer products. Despite the growing attention lead poisoning has attracted over the last 40 years in developed countries, lead poisoning has not received the same level of concern or attention in some developing nations [13,16,26].

Numerous studies have depicted a relationship between lead exposure and intellectual deficits in children [18,20,24]. Clinical studies have suggested that lead exposure can affect brain development, resulting in cognitive deficits in early childhood [2,26]. Research in rats has shown that lead exposure can effect the development of brain structures including the hippocampus, which has been widely associated with memory function [1,14,15]. As fetal and infant brains are in a rapid state of development and may be more vulnerable to environmental toxicant, exposure to relatively minor levels of environmental lead may lead to impaired cognitive functions following development.

In 1969, Cherkin noticed a young chick's tendency to peck at small bright objects, such as beads and developed a one-trial passive avoidance learning task to investigate memory [6]. Ever since, the investigation of a chick's ability to complete this task has become a powerful model system in which memory function can been evaluated [23].

In addition, the chick provides other advantages in the investigations of fetal compromise with respect to other animal models, such as a rat model. As fetal development in rats is dependent on the materno-placental unit and experimentation often entails manipulation to the mother [11], models of post-natal rats may be confounded by responses from the mother and/or the placenta to the external perturbations. The chick model eliminates maternal influences because the embryo develops independently of the hen and allows experimentation without these confounding issues. Furthermore, chicks, being precocial, are able to perform a variety of cognitive functions soon after they hatch. Therefore, the one-trial passive avoidance learning paradigm provides a useful model to assess the ability of chicks incubated under different prenatal conditions to form memory. It has been used to study the effect of prenatal hypoxia, morphine exposure, alcohol exposure and malnutrition on memory consolidation after hatching [3,4,21,22].

In the present study, the one-trial passive avoidance learning paradigm was used to investigate if prenatal lead exposure alters the capacity for learning and memory in nascent chick offspring. Lead was injected into the air space of chick eggs and the limiting effect of prenatal lead exposure on memory consolidation in day old chicks was measured.

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Fertilized chick eggs with embryos were purchased from a local hatchery. Eggs were incubated at 38 °C and about 60% relative humidity in a forced-draught incubator with automatic egg turning every 1 h. Eggs were weighed, numbered and assigned to 7 groups (n = 46–47/group) in order to match the egg weights of the seven groups. Groups of embryonic eggs were given injections once daily from E9 to E16 (8 days) of either lead acetate or sodium acetate at the respective doses (16.5 mg/kg, 11 mg/kg, 5.5 mg/kg) into the airspace of the eggs, while the control group received no injections over the same period. These injection doses and administration times were selected according to previously established protocols [10,12]. One day prior to treatment administration, holes were drilled in the eggshells for injections into the airspace. The syringe needle was inserted to a depth of 1 cm, and the hole was resealed with wax after injection [12]. Injection volumes used were two microliters of solution per each gram of egg weight (average solution volume = 118 μl). These volumes are considered to be a negligible burden on the egg [10,12]. The accumulative dosage of the eight injections was 132 mg/kg, 88 mg/kg, and 44 mg/kg for the respective treatment groups. The lead plasma levels (~20 μg/dl) in chicks at day 4 after hatching are within the levels found in many children worldwide [10,12].

The chicks hatched normally on day 21 (post-hatch age was counted as day 1) and hatching was considered to be the day the chicks completely came out from the eggshells. After the chicks hatched, they were transferred to a heated brooder. Chicks were weighed once they were completely dry.

The one-trial passive avoidance learning task exploits the spontaneous tendency of chicks to peck at objects in their immediate environment. Chicks were trained using a variant of the passive avoidance training procedure described by Crowe and Hale [9]. The task involved three components: pre-training, training and retention trials. Twenty-four hours post-hatch, the chicks were placed, in pairs, into 20 cm × 20 cm × 25 cm training pens, each maintained at 28–30 °C and illuminated by an overhead 25 W light bulb. Fresh water and food was provided *ad libitum*. Chicks were left to settle in the boxes for at least 30 min before any testing procedures began. One chick in each pair was marked with a small black stripe on its head for identification purposes during the data recording. At pre-training, chicks were presented with a glass bead (2 mm in diameter). The bead was dipped in water to encourage the chicks' natural pecking response. This procedure was repeated after a period of about 20 min. Following the second pre-training with the glass bead, the chicks were presented with a red bead (5 mm in diameter). Again, the bead was dipped in water and was then presented for 10 s. The number of pecks was recorded. The training trial involved presentation of a red bead visually identical to that used in pre-training. The bead was dipped in 100% (v/v) of the bitter-tasting methylanthranilate (MeA). The chicks typically gave a disgusted reaction after pecking at the aversive bead: shaking their heads, closing their eyes and occasionally wiping their beaks on the floor of the box. The bitter-tasting bead was presented to the chicks for 10 s. Chicks that failed to peck at the training bead were excluded from the subsequent analysis. The retention trial was conducted at 5 min (Short-Term Memory (STM)) or 30 min (Intermediate-Term Memory (ITM)) or 120 min (Long-Term Memory (LTM)) after training [22]. Chicks were again presented with a red bead identical to the one in the pre-training procedure. The number of pecks was recorded. An avoidance ratio (AR) was calculated: the number of pecks at the red pre-training bead divided by the number of pecks at the red retention trial bead plus the number of pecks at the red pre-training bead (i.e., AR = pecks pre/pecks pre + pecks retention trial).

Eight chicks from each experimental group were used for the analysis of lead concentration in the brain. After completing the one-trial passive avoidance learning test, chicks were deeply anesthetized with 2.5% sodium pentobarbital at an intraperitoneal dose of 80 mg/kg and then were euthanized by decapitation. After decapitation, the whole brain was isolated, placed on an ice-cold plate, rinsed with ice-cold sterile saline and dissected using a plastic mould and a razor blade as diagrammatically represented in Fig. 1. Section 2 of the dissection contains the intermediate medial mesopallium (IMM) and medial striatum (MSt) which are the main functional regions associated with learning in the one-trial passive avoidance task [8]. Brain matter from section 2 was used for the determination of lead concentration in each sample. Brain tissue was transferred to Teflon vials, dried at 65 °C to a constant weight, digested for 8 h in 3 ml hot 16N HNO_3. The HNO_3 was evaporated until the brain extract was a dry powder, and dissolved in 1N HNO_3 for analysis. The lead in each sample was measured using inductively coupled plasma optical emission spectrometry (ICP-OES) [25].

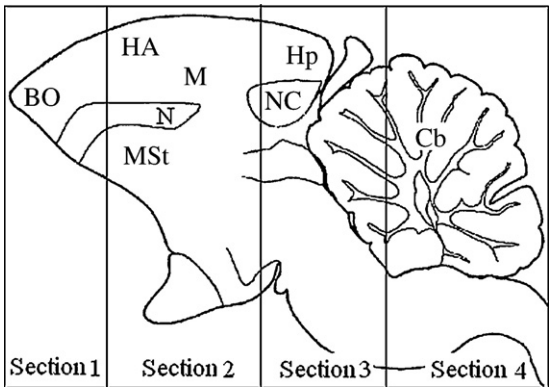

Fig. 1. Sagittal section of the chick brain (adapted from Youngren and Phillips, 1978 [29]) demonstrating the coronal sections made using the mould and razor blade. N, nidopallium; Cb, cerebellum; MSt, medial striatum; Hp, hippocampus; M, mesopallium; HA, apical part of the hyperpallium.

All data were analyzed using the statistical package for social sciences (SPSS10.0). A one-factor ANOVA followed by Dunnett's post hoc tests was performed on the data. Data are presented as mean ± S.E.M. Significance was accepted at $p \leq 0.05$.

There was a significant difference ($p < 0.001$) in the hatching success and the hatch weight between the lead acetate treatment groups (11 mg/kg, 16.5 mg/kg) and the sodium acetate groups treated at similar concentrations (11 mg/kg, 16.5 mg/kg; see Table 1). However, no significance was found between the lead acetate (5.5 mg/kg) treatment group, the sodium acetate treatment group (5.5 mg/kg), and the control group ($p > 0.05$, see Table 1). The average length of incubation period prior to hatching was not significantly different between any of the treatment groups (chicks injected with either sodium acetate or lead acetate) and the control group ($p > 0.05$, see Table 1).

The higher doses of lead (16.5 mg/kg and 11 mg/kg) greatly reduced the hatching success of chicks in those groups. Unfortunately, the numbers of successfully hatched chicks from these two groups were not great enough for the one-trial passive avoidance learning experiment to be completed. Treatment of eggs with the 5.5 mg/kg doses of lead produced no grossly observable deficits in chick survival, weight or morphology.

The lead acetate (5.5 mg/kg) and sodium acetate (5.5 mg/kg) treated and control chicks were observed in the one-trial passive avoidance learning task. No significant difference was observed in the number of pecks at the red bead during pre-training among the three groups (data not shown). The spontaneous tendency of young chicks to peck at objects was not affected by prenatal treatment of either lead acetate or sodium acetate. All three groups of chicks remembered the aversive taste of the bead at the 5-min