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Effects of low level lead exposure on associative learning and memory in the rat: Influences of sex and developmental timing of exposure

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HIGHLIGHTS

- Developmental lead exposure modifies associative learning and memory.
- Developmental time and size of lead exposure differentially modify associative memory.
- Outcomes of developmental lead exposure on associative memory differ by sex.

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ABSTRACT

Lead (Pb) exposure during development impairs a variety of cognitive, behavioral and neurochemical processes resulting in deficits in learning, memory, attention, impulsivity and executive function. Numerous studies have attempted to model this effect of Pb in rodents, with the majority of studies focusing on hippocampus-associated spatial learning and memory processes. Using a different paradigm, trace fear conditioning, a process requiring coordinated integration of both the medial prefrontal cortex and the hippocampus, we have assessed the effects of Pb exposure on associative learning and memory. The present study examined both female and male long evans rats exposed to three environmentally relevant levels of Pb (150 ppm, 375 ppm and 750 ppm) during different developmental periods: perinatal (PERI; gestation—postnatal day 21), early postnatal (EPN; postnatal days 1–21) and late postnatal (LPN; postnatal days 1–55). Testing began at postnatal day 55 and consisted of a single day of acquisition training, and three post training time points (1, 2 and 10 days) to assess memory consolidation and recall. All animals, regardless of sex, developmental window or level of Pb-exposure, successfully acquired conditioned-unconditioned stimulus association during training. However, there were significant effects of Pb-exposure on consolidation and memory recall at days 1–10 post training. In females, EPN and LPN exposure to 150 ppm Pb (but not PERI exposure) significantly impaired recall. In contrast, only PERI 150 ppm and 750 ppm-exposed males had significant recall deficits. These data suggest a complex interaction between sex, developmental window of exposure and Pb-exposure level on consolidation and recall of associative memories.

1. Introduction

Lead (Pb) exposure during development impairs a variety of cognitive processes in children including learning, memory, attention, and various aspects of executive function, and, these deficits can persist into adulthood (Cecil et al., 2008; Cecil and Kos, 2006; Mazumdar et al., 2011; Nigg et al., 2010; Surkan et al., 2007).

The cognitive/behavioral effects of Pb exposure have been modeled in numerous animal studies, with the majority of studies focusing on hippocampal-associated spatial learning and memory processes in rodents, most often using the Morris water maze (ex., Jett et al., 1997; Toscano and Guilarte, 2005). Although these studies have provided useful information on the effects of Pb on hippocampus-associated learning and memory, the water maze task may not be optimal for detecting more subtle behavioral disturbances as might occur with lower level exposures to Pb (Anderson et al., 2012; Jett et al., 1997). Other studies have examined the effects of low level developmental Pb exposures (i.e., blood Pb levels $<10\ \mu g/dl$) on frontal cortex-associated attention

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and impulsivity problems using fixed-interval/fixed ratio testing or performance on a delay discounting task (Brockel and Cory-Slechta, 1998; Weston et al., 2014). While these studies have provided important information regarding the neurotoxic effects of low level developmental Pb exposure on clinically relevant cognitive processes and brain neurochemistry, studies using other experimental paradigms are also needed in order to better understand some of the more complex cognitive/behavioral issues reported in Pb-exposed children.

Children exposed to low levels of Pb experience a variety of behavioral disorders that impact cognitive performance and academic achievement (Bellinger, 2008a,b; Lanphear et al., 2005; Needleman, 2004; Zhang et al., 2013). Of the numerous adverse behavioral outcomes from developmental Pb exposure, anti-social and aggressive behaviors are among the most common and troublesome. Although anti-social and aggressive/violent behavior can be attributed to a variety of social factors, increased frequency of anti-social and aggressive behaviors as well as other behavioral disorders such as oppositional defiant behavior and anxiety have been shown to be related to developmental Pb exposure (Carpenter and Nevin, 2010; Chiodo et al., 2004; Liu et al., 2014; Nevin, 2007; Nigg et al., 2008). There has been little basic research on these aspects of developmental Pb neurotoxicity and their relationship to disturbed cognitive outcomes. Alterations in neural circuitry or neural functioning consequent to developmental Pb exposure that result in maladapted behavioral reactions to perceived threats or inappropriate fear responses in non-threatening situations may play a role in at least some of the behavioral/cognitive abnormalities seen in Pb-exposed populations.

Fear conditioning is a valuable tool with which to study the neural systems involved in the acquisition, consolidation, recall and extinction of memories (Gilmartin and Helmstetter, 2010). In particular, trace fear conditioning, in which a neutral conditioned stimulus (CS) is paired with an aversive unconditioned stimulus (US) and a "trace" interval of several seconds is incorporated between the CS and US, has shown the importance of attentional mechanisms and the role of the prefrontal cortex in associative learning and memory (Gilmartin and Helmstetter, 2010; Han et al., 2003). Using fear conditioning tasks, a direct role for the prefrontal cortex in associative memory storage has been suggested (Runyan et al., 2004) as have potential epigenetic mechanisms controlling associative learning and memory formation (Lubin et al., 2008; Miller et al., 2010). Although effects of Pb exposure on fear conditioning have been examined previously, those studies used only males with blood Pb levels in excess of 30 μg/dl (Jaako-Movits et al., 2005; Salinas and Huff, 2002) and none used a trace conditioning paradigm. Understanding effects of developmental Pb exposure on trace fear conditioning could potentially provide important information on how Pb interferes with the functioning of prefrontal circuits involved in complex associative behaviors. Such studies could also provide insight into how developmental Pb exposure may adversely influence the subjective assessment of and behavioral response to threat and the learning, memory, and expression of fear responses in threatening and non-threatening situations (Gilmartin et al., 2014). Dysfunction in prefrontal-hippocampal-amygdala circuits consequent to developmental Pb exposure could at least in part contribute to various forms of behavioral pathology that have been associated with Pb exposure and trace fear conditioning paradigms may be valuable tools with which to study this in the context of associative learning and memory.

We have recently reported significant differences in hippocampus-based spatial learning and memory in males and females following developmental Pb exposure (Anderson et al., 2012). The developmental period during which Pb exposure occurs can also significantly influence cognitive and neurobiological outcomes

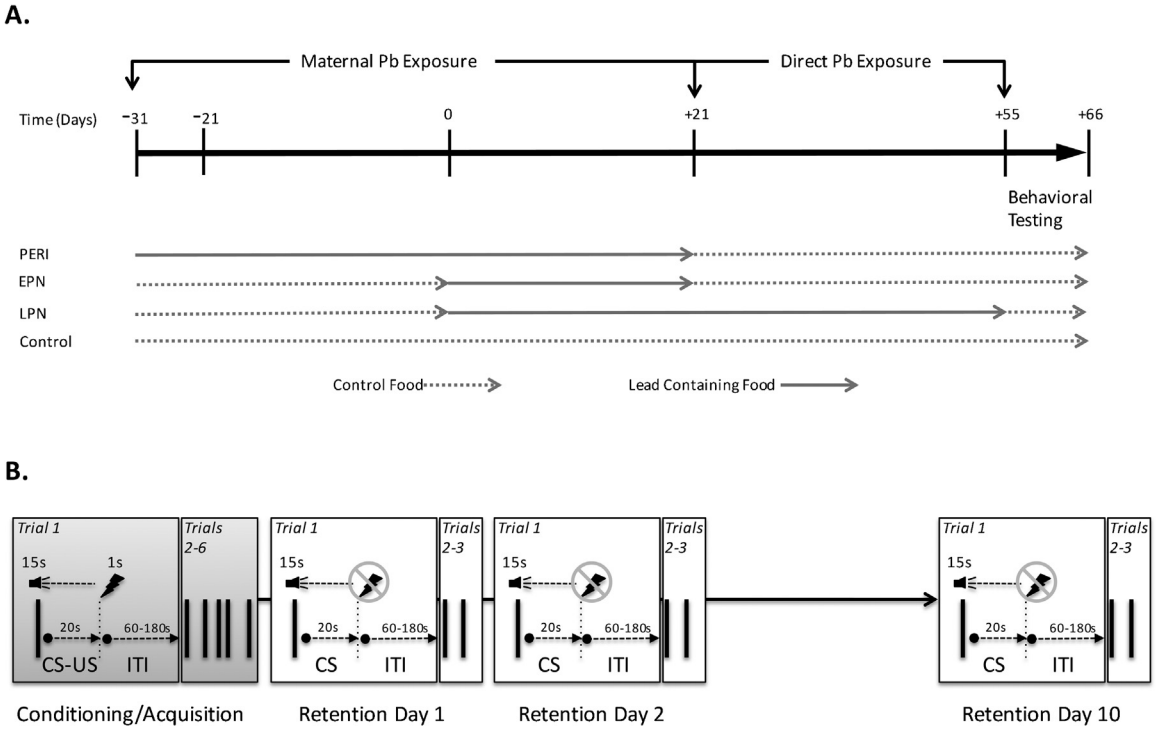

Fig. 1. (A) Timelines for lead exposure. All animals were either exposed to lead (Pb) containing chow (RMH1000 containing 150, 375 or 750 ppm lead acetate) and or control chow (RMH1000). Timing and duration of Pb exposure was either perinatal (PERI), Early postnatal (EPN) or Long-term postnatal (LPN), as shown. All animals were behaviorally assesssed on a trace fear conditioning test at postnatal days 55–66. (B) Fear conditioning experimental design. All animals underwent 1 day of initial training using a pseudo-random CS (tone; 15 s duration)-US (shock, 1 s duration) combination (6 paired tones and shocks) separated by pseudo-random inter-trial intervals (ITI). The animals were then place in a novel context (novel visual, olfactory and tactile environment as described in detail in the methods) at 1, 2 and 10 days post training and presented with 3 cues (tone) separated by pseudo-random ITI's with no subsequent shock, and the degree of freezing behavior was recorded (Anymaze, Stoelting Co.).