CHRONIC DEVELOPMENTAL LEAD EXPOSURE REDUCES NEUROGENESIS IN ADULT RAT HIPPOCAMPUS BUT DOES NOT IMPAIR SPATIAL LEARNING

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SUMMARY

The dentate granule cell (DG) layer of the hippocampal formation has the distinctive property of ongoing neurogenesis that continues throughout adult life. Although the function of these newly generated neurons and the mechanisms that control their birth are unknown, age, activity, diet and psychosocial stress have all been demonstrated to regulate this type of neurogenesis. Little information on the impact of environmental insults on this process has appeared to date. Developmental lead (Pb) exposure has been well documented to impair cognitive function in children and animals and reduce activity-dependent synaptic plasticity in the hippocampus of rodents. Therefore, we examined the effects of this classic environmental neurotoxicant on hippocampal-dependent learning and adult neurogenesis in the hippocampus. Pregnant rats were exposed to a low level of Pb-acetate (0.2%) via the drinking water from late gestation (GD16) until weaning on postnatal day 21 (PN21). At weaning, half of the Pb-exposed animals were weaned to control drinking water and the remainder were maintained on Pb water until termination of the study. Animals were paired-housed and on PN75 were administered a series of injections of a thymidine analog bromodeoxyuridine (BrdU), a marker of DNA synthesis that labels proliferating cells and their progeny. At 12 hr intervals for 12 days, rats received an ip injection of BrdU (50 mg/kg). Subjects were sacrificed and perfused 24 hr and 28 days after the last injection. Spatial learning was assessed in an independent group of animals beginning on PN110 using a Morris water maze. No Pb-induced impairments were evident in water maze learning. Immunohistochemistry for the detection of BrdU-labeled cells was performed on 40 µm coronal sections throughout the hippocampus. Continuous exposure to Pb (Life) reduced the total number of BrdU-positive cells at 28 days without affecting the total number of labeled cells evident 24 hr after the last injection. No differences in the number of
progenitor cells labeled or surviving were seen between control and treated animals whose Pb exposure was terminated at weaning. Double labeling with BrdU and the glial specific marker, glial acidic fibrillary protein (GFAP) indicated that the bulk of the surviving cells were of a neuronal rather than a glial phenotype. These data reveal that chronic low level Pb exposure reduces the capacity for neurogenesis in the adult hippocampus. Despite deficits in synaptic plasticity previously reported from our laboratory (e.g., Gilbert, 1998), and now structural plasticity, no significant impact on spatial learning was detected.

KEYWORDS: Dentate gyrus, adult neurogenesis, lead, Pb, in vivo, hippocampus, rat, neurotoxicity, learning and memory, spatial learning, Morris water maze
INTRODUCTION

Encephalopathy, coma, convulsions, and death have been well documented to result from severe lead (Pb) poisoning in children (Goyer, 1990). At lower environmentally relevant levels of exposure, however, subtle long-term impairments in intellectual function and affective behavior can occur in the absence of readily apparent aberrations in brain morphology or gross neurological deficits. Of most concern among the effects of low level Pb exposure is the occurrence of reduced cognitive capacity in children exposed early in life (Bellinger et al., 1991; Riess and Needleman, 1992).

Despite intensive research efforts spanning several decades, the cellular mechanisms underlying the clinical manifestation of low level Pb-induced neurotoxicity have remained elusive. Alterations in the properties of, glutamatergic, cholinergic and dopaminergic neurotransmitter function and signal transduction have been reported (see reviews by Lasley and Gilbert, 2000; Cory-Slechta, 1995). Several studies have indicated impairment in the induction, expression and maintenance of long-term potentiation (LTP) in the hippocampus of animals exposed to Pb during the early postnatal period (e.g., Altmann et al., 1993; Gilbert et al., 1996; 1999a; b; Gilbert and Mack, 1998; Ruan et al., 1998) and have suggested that such deficits may underlie some of the cognitive impairments associated with early Pb exposure. Reduced capacity to support LTP in the dentate gyrus of Pb-exposed animals is associated with reductions in hippocampal glutamate release (Lasley and Gilbert, 1996; 2002; Lasley et al., 1999), binding properties of the N-methyl-d-aspartate (NMDA) antagonist, MK-801, in the hippocampus (Lasley and Gilbert, 1999; Lasley et al., 2001), and alterations in gene and protein expression of NMDA receptor subunits in the dentate gyrus (Guilarte et al., 2000; Nihei and Guilarte, 2002).
In addition to a propensity for LTP, the hippocampus has received considerable attention recently for another form of plasticity, namely, neurogenesis in the mature brain. This phenomenon, initially reported by Altman and Das (1965), refers to the birth of new neurons in the dentate gyrus that occurs throughout the lifetime of the animal. Neural stem cells are abundant in the subgranular zone of the dentate gyrus of the hippocampal formation of the adult and are capable of dividing and differentiating into neurons. These cells migrate into the granule cell layer, express neuronal phenotypes, receive synaptic contacts, make functional synaptic connections, and exhibit activity-dependent LTP (van Praag et al., 2002; Wang et al., 2000). It has been purported that dynamic changes in neural stem cells may contribute to underlying substrates of learning and memory (Gould et al., 1999; Shors et al., 2001; 2002). The presence of stem cells in adult brain may also serve as a reserve of neural cells to replace cells that die as the result of various injuries and diseases throughout the lifetime of the animal.

A number of factors have been identified that influence neurogenesis in the adult hippocampus. Genetics and prenatal viral infections can reduce the capacity for neurogenesis in the adult (Sharma et al., 2002; Kempermann et al., 1997). The birth and survival of new cells in the dentate gyrus is increased by environmental enrichment, reduced caloric intake, physical activity, hippocampal-dependent learning, and both physiological activation (e.g., neuronal activity) or pathological insult (e.g., seizure, stroke) (Derrick et al., 2000; Gould et al., 1999; Kempermann et al., 1998; 2002; Leuner et al., 2000; Liu et al., 1998; Mattson, 2000; Parent et al., 1997; Scharfman et al., 2000; Shors et al., 2001). The birth of new cells is decreased by stress, age, and chronic exposure to drugs of abuse including opiates and alcohol (Eisch et al., 2000; Gould et al., 1997; Herrera et al., 2003; Kempermann et al., 2002; Kuhn et al., 1996;
Nixon and Crews, 2002). To date, little information is available on the impact of exposure to environmental contaminants on this phenomenon. Given the deleterious effects of developmental Pb exposure on synaptic plasticity in the form of LTP, we sought to determine the impact of chronic exposure to Pb on this form of structural plasticity that is prominent in the hippocampus. We also examined the performance of Pb-exposed animals on spatial learning in a task believed to be dependent upon the hippocampus. We report no effect of developmental Pb exposure on spatial learning in a Morris water maze or on neural cell proliferation. However, a reduced capacity for survival of newly generated granule cells in the dentate gyrus of adult rats was observed in rats chronically exposed to Pb.

**METHODS**

**Subjects.** Pregnant Long Evans rats were obtained from the Charles River (Raleigh, NC) on gestational day (GD) 14. On GD16 dams were placed on 0.2% Pb acetate in the drinking water, while controls were administered deionized water. Both groups of animals were maintained on NIH-07 chow (Ziegler Bros., Gardners, PA) to ensure consistent levels of mineral intake. At parturition litters were culled to 8 pups, retaining the maximal number of males per litter. On postnatal day (PN) 21 offspring were weaned, housed in a colony room on a 12:12 light:dark schedule, and permitted free access to food and water. Animals were housed in pairs in plastic cages with pine shavings as bedding. Half of the animals from each Pb-exposed litter was maintained on a 0.2% Pb-treated water supply until sacrifice (Pb-Life). The remainder were placed on deionized water at weaning (Pb-Wean). All offspring from each control litter were maintained on deionized water at weaning (Control).
**BrdU Dosing.** Beginning on PN75 all animals were administered 50 mg/kg BrdU (Sigma, St Louis, MO) ip, twice daily at 12 hr intervals for 12 consecutive days. A variety of regimens of BrdU administration have been reported with a wide range of dosing parameters. There are proponents of low doses to avoid BrdU-induced damage or higher doses to maximize visualization of dividing cells. The number of cells labeled by a given injection of BrdU is limited by the bioavailability of BrdU (half-life ~ 2 hours) and the number of cells in the S-phase of the cell cycle during this brief period. Typically studies designed to identify changes in neurogenesis due to age, lifestyle (e.g., exercise), or long-lasting treatment manipulations (e.g., chronic ethanol exposure, environmental housing, epilepsy, viral infection) have adopted dosing regimens that span over several days (Cameron and McKay, 2001; Herrera et al., 2003; Kempermann et al., 1998; Sharfman et al., 2000; Sharma et al., 2002). Following the rationale of Kempermann et al. (1998) and Herrera et al. (2003), we selected a BrdU regime that allowed us to reliably detect an adequate number of cells that underwent division without inducing damage.

One male from each litter was perfusion-fixed through the aorta with 4% paraformaldehyde 24 hours after the last dose of BrdU (n=5/treatment group). The remaining male from each litter was perfusion fixed 27 days later (n=5/ treatment group) such that each group had only a single representative from any litter. Figure 1 outlines the timing of Pb and BrdU exposure conditions.

**Immunohistochemistry.** The brain was removed following perfusion of the animal and stored in
4% formaldehyde for several days prior to being transferred to cryoprotectant, and subsequently sectioned at 40µm using a freezing microtome. Every section throughout the hippocampus was saved in 5 consecutive bins such that each bin contained sections throughout the hippocampus at 200µm intervals.

In preparation for BrdU immunocytochemistry, free-floating coronal sections were initially incubated in 50% formamide dissolved in 2x SSC (0.3M NaCl, 0.03M sodium citrate) followed by 2N HCl to denature the DNA (Parent et al., 1997). Immunocytochemical staining was performed using the methods of Goodman and Sloviter (1993). Briefly, the sections were washed in Tris buffer (pH 7.6) followed by incubation in 1% hydrogen peroxide to remove endogenous peroxidase activity. Sections were then washed sequentially in Tris followed by Tris A (0.1M Tris plus 1% triton X 100) and then Tris B (0.1M Tris, 1% triton X 100, 0.05% BSA). The sections were then incubated in anti BrdU antiserum (1:1000 dilution, monoclonal, Boehringer Mannheim, Indianapolis, IN) at 4°C for 48 hr. On the second day of processing the sections were incubated in biotinylated secondary antiserum (horse antimouse, dilution 1:400, Vector Labs, Burlingame, CA) followed by avidin-biotin complex (ABC Elite -Vector, 1:1000 dilution), and visualized with diaminobenzidine as the chromogen, which was enhanced with NiCl. Stained sections were mounted on glass slides, dehydrated and coverslipped.

To indirectly ascertain the phenotype of newly born cells, BrdU-stained sections were double-labeled with an antibody recognizing glial fibrillary acidic protein (GFAP), a protein selectively localized in glial cells. Free-floating BrdU-stained sections, processed as described above, were incubated in anti-GFAP antiserum (1:1000 dilution, monoclonal, Boehringer Mannheim, Indianapolis, IN) at 4°C for 48 hr and visualized with Vector NovaRed (Vector
Labs, Burlingame, CA) as the chromogen.

**Cell Counting.** BrdU-stained sections through the dorsal hippocampus were examined from each animal and BrdU-labeled cells from both the upper and lower blades of the dentate gyrus of both hippocampi were counted in a blinded fashion. Serial sections at 200 µm intervals falling between Plates 31 and 37 from Paxinos and Watson (1998) were included and counts generated from 3-5 sections were averaged for each animal. When labeled cells were found in clusters the focal plane was changed to maximize the ability to distinguish individual cells. Granule cells are born at the base of the granule cell layer and migrate towards the molecular layer as they mature (Cameron et al., 1993). Therefore, only BrdU profiles directly in the granule cell layer or touching the granule cell layer were included in the counts, to the exclusion of BrdU-stained cells residing in the dentate hilus and molecular layer. While this practice should increase the likelihood that only newly born granule cell neurons were counted, some of the BrdU-labeled cells could be glial in origin. This possibility was assessed by double labeling BrdU-stain sections with the glial cell marker, GFAP as described above.

**Behavioral Testing:** Behavioral testing was performed in 2 independent cohorts of animals beginning on PN110. In the first group litters were exposed to 0 (n=8) or 0.2% (n=8) Pb in a similar fashion to that described above and removed from Pb water on PN21. A second group of control (n=8) and Pb-exposed (n=8) litters were exposed developmentally as described and maintained on Pb throughout life. One male from each litter and exposure condition (Control, Pb-Wean and Control, Pb-Life) was tested for performance in a Morris water. Animals were
placed in a quiet holding room adjacent to the test room for a minimum of 30 minutes prior to testing. The water maze consisted of a white circular galvanized tank with a diameter of 165 cm, filled with tap water adjusted to 27°C and made opaque by the addition of white non-toxic tempura powdered paint. Four equally spaced locations around the edge of the pool were defined as start points, and divided the pool into 4 equally sized quadrants. A circular acrylic escape platform, 10 cm in diameter was placed 2 cm below the surface of the water in the middle of one of the 4 quadrants of the pool. The pool was placed in a 2.5 X 2.5 meter square room containing invariant spatial stimuli. A video camera suspended from the ceiling above the middle of the tank permitted the observer to monitor the animal’s behavior on a monitor placed in one corner of the room. Animals were tested for 2 daily trials, each trial separated by approximately 3-5 minutes for 15 consecutive days. Animals were placed into the tank, facing the wall of the pool, and allowed to circumnavigate the pool in search of the escape platform for a maximum of 60 seconds. On each day the start points used for each trial varied in a pseudo-random sequence such that no two trials on the same day commenced from the same start point. Escape latency to reach the escape platform was recorded and the animals permitted 15 seconds to rest on the platform before removal from the tank. If an animal failed to locate the platform within 60 seconds, it was guided to the platform by the experimenter, placed upon it for 15 seconds, and assigned a latency score of 60 seconds for that trial. A series of probe trials was inserted on the first trial on test days 3, 6, 9, 12 and 15 in which the platform was removed and animals allowed to swim freely for 60 seconds. The platform was reinserted at the end of this period to avoid extinction of the response and animals were permitted to rest on it for 15 seconds. The percentage of time animals spent in each quadrant of the pool was recorded for each probe trial.
Escape latencies on probe trial days were based on data from the 2nd trial only. Cue testing was conducted on the final trial of day 15. Under this condition, the platform was visible resting above the water level and a glove identical to that worn by the experimenter was suspended above the platform. Animals were placed in the pool as on previous trials and latency to reach the visible platform was recorded.

**Statistical Analysis:** The mean number of cells/hippocampus was calculated group differences in the mean number of BrdU-positive cells at the 24-hour (cell proliferation) and 28-day sacrifice (survival) times were evaluated using analysis of variance (ANOVA). Mean contrast tests were performed when significant effects were observed (Tukey HSD, p<0.05). For behavioural assessments, no differences were observed in control groups from the Pb-Wean vs the Pb-Life cohorts, so control data were combined to facilitate comparison across groups. One animal in the Pb-Wean condition developed an abscess on the back of the head and was removed from further testing. Repeated measures ANOVA was used to assess mean latency across days and probe trial percentage scores. One-way ANOVAs were used to assess cue learning latencies and swim speeds.

**RESULTS**

This developmental Pb dosing regimen has repeatedly produced Pb blood levels of between 35-40μg/dl in adult animals maintained on Pb (Gilbert et al., 1996; 1999b; Lasley et al., 2002; Lasley and Gilbert, 1996). Pb blood concentrations return to background control levels in subjects removed from Pb at weaning (Gilbert et al., 1999a). No significant alterations in body weight were seen at weaning or in adulthood as a result of Pb exposure.
Neurogenesis: A large number of darkly stained BrdU-positive cells were evident in the cell body layer of the dentate gyrus in all three groups of animals sacrificed 24 hours after the last of 24 BrdU injections (Figure 2a-2c). The mean number of BrdU-positive profiles present in the dentate gyrus was comparable across all groups in animals sacrificed 24 hours after the last BrdU treatment (Figure 2d), indicating that Pb exposure did not alter the proliferation of progenitor cells. As such, it is unlikely that the presence of Pb in the tissue interfered with the incorporation of BrdU into the nucleus. In contrast, chronic Pb exposure did reduce the number of labelled cells present in animals sacrificed 28 days after the last administration of BrdU (Figure 3c) compared to controls (Figure 3a). A small, but nonsignificant reduction was also seen following a more restricted period of Pb exposure that terminated at weaning (Figure 3d). ANOVA revealed a significant effect of Treatment [F(2,12)=10.7, p<0.0021] that was limited to the chronic Pb exposure group (Life, Tukey’s, p<0.05). These data indicate the chronic Pb exposure reduced the survival of newly generated cells in the adult dentate gyrus.

The morphological profile of BrdU-labelled cells and their location within the granule cell layer are consistent with that of neurons as can be seen at higher magnification in Figure 4. The BrdU-positive cells are incorporated into the granule cell layer, and unlike glial cells, the nucleus is large and fills the bulk of the cytoplasm of the cell soma. Also, a comparison of the BrdU staining in Figure 3 to the staining in Figures 3 and 4 reveals that at the 28 day time point, the BrdU labeled cells have moved up within the granule cell layer which is the typical behavior of granule cells as they mature (Cameron et al., 1993). Double labeling sections with an antibody against GFAP, a protein selectively expressed in glial cells, revealed that few if any of the BrdU-
labeled cells stained for GFAP (Figure 5). Under high power magnification, the triangular shaped somata of astrocytes expressing GFAP and residing below the granule cell layer in the hilar region are readily apparent. Their position and morphology contrast sharply with the round soma of BrdU-positive cells within the granule cell layer. Comparing a Nissl stained section in Figure 5e of granule cells and astrocytes to the double labeling of BrdU and GFAP in Figure 5f clearly shows the distinction between these cell types and supports the premise that the surviving cells staining positively for BrdU represent granule cells.

**Behavioral Testing:** No differences between control and Pb-exposed groups were evident in acquisition of the Morris water maze (Treatment F (2,28)=0.45 p>0.64). Learning, assessed by the reduction in latency to find the hidden platform over days, was clearly evident in all groups (Figure 6a, Day F (13,364)=32.91, p<0.0001). Under cued conditions, all animals located the platform in less than 10 seconds demonstrating no group differences in visual function or motoric competence. Probe trials in which the platform was removed and animals allowed to swim for 60 seconds were interspersed throughout the acquisition phase as another dependent measure of learning. Consistent with latency measures, probe trials revealed increasing time spent searching in the correct quadrant over days in all animals (F(4,236)=22.29, p<0.0001), with no difference between groups (Figure 6b, F(2,59)=0.01,p>0.99).

**DISCUSSION**

The current findings reveal a Pb-induced deficit in the survival of newly generated cells within the dentate gyrus of the adult hippocampal formation. This regimen of chronic
developmental Pb exposure did not result in overt toxicity based on absence of physical abnormalities or alterations in body weight or mortality, nor were impairments in standard test of spatial learning observed. No differences in the number of cells born were detected in animals sacrificed 24 hours after cessation of BrdU dosing indicating that cell genesis was unaffected, and that the presence of Pb in the brain did not interfere with the incorporation of BrdU into the nucleus of dividing cells. A reduction in number of BrdU-positive cells 1 month after labeling was observed in animals chronically exposed to Pb from just prior to birth and continuing on throughout the postnatal period. Exposure to Pb that was limited to the preweaning period did not significantly alter the survival of newly generated cells. Precursors in the subgranular zone migrate into the dentate granule cell layer where the majority (85-95%) ultimately acquire morphological characteristics of granule cells and express neuron-specific markers (Farmer et al., 2004; Okano et al., 1993; Cameron et al., 1993). We believe that cells with BrdU profiles at the 28-day time point in the present study represent neurons due to the size of the labeled nucleus, their position within the dentate granule cell layer, and their absence of GFAP staining.

A number of organismal, experiential and environmental factors have been shown to influence the process of adult neurogenesis. It is unlikely that age, diet or level of physical activity differentially affected neurogenesis in control and Pb-exposed animals in the present study. Neuronal activation, by stimulation to induce LTP or seizure activity, augments neurogenesis and cell survival (Derrick et al., 2000; Parent et al., 1997; Scharfman et al., 2000). Blockade of NMDA receptor activation increases cell proliferation (Nacher et al., 2003; Gould et al., 1997). Pb-exposed animals have higher thresholds for induction and a reduced capacity to support LTP (Gilbert et al., 1996; 1999a; b). The maintenance phase of LTP in the dentate gyrus
of the intact animal that occurs over days and weeks post-induction is also significantly reduced in Pb-exposed animals (Gilbert and Mack, 1998). Deficits in LTP are correlated with reductions in calcium-dependent glutamate release within the hippocampus (Lasley et al., 1999; Lasley and Gilbert, 2002), and enhanced binding of the NMDA antagonist MK-801 (Lasley et al., 2001). Alterations in NMDA receptor density as a function of chronic Pb exposure may reflect an upregulation of NMDA receptors in response to reduced glutamate release (Lasley and Gilbert, 2000; Lasley et al., 2001). Additionally, alterations in mRNA expression of glutamate receptors have also been reported to accompany developmental Pb exposure (Guilarte et al., 2000; Nihei and Guilarte, 2002). Thus, the effects of Pb exposure on LTP and survival of newly generated granule cells in the adult hippocampus may derive in part from the actions of Pb on neuronal activation and glutamate-mediated synaptic transmission.

The reduction in survival of newly generated cells in the dentate gyrus was limited to chronically exposed animals maintained on Pb throughout life. BrdU-positive cells were slightly (~20%) but not significantly reduced in number in animals receiving a more limited exposure to Pb. This suggests that the continued presence of Pb may be necessary for the effects on neurogenesis. In studies examining Pb effects on LTP and neurotransmitter release, chronic exposure produced the largest impairments, whether exposure began in late gestation or at weaning (Gilbert et al., 1999a; Lasley et al., 1999). Animals exposed only until weaning and tested as adults when Pb in blood and brain had returned to background levels, revealed a deficit in LTP that was smaller in magnitude and limited to the EPSP component of the compound field potential. Similarly, Pb-induced reductions in calcium-dependent glutamate release in hippocampus were of lower magnitude in animals whose Pb exposure was limited to the
preweaning period, relative to those maintained on Pb throughout life (Lasley et al., 1999). These data suggest that if the Pb-induced impairments in glutamate synaptic transmission are responsible for reduced neurogenesis, additional studies with larger sample sizes yielding greater statistical power may also reveal deficiencies in neurogenesis in Pb exposures limited to the perinatal period.

Environmental conditions can dramatically affect adult neurogenesis (Kempermann et al., 1998; Rochefort et al., 2002). Isolated rearing and impoverished housing reduce the proliferation and survival of newly generated neurons, whereas dietary restriction and voluntary exercise enhance cell survival (Farmer et al., 2004; Lee et al., 2002; Mattson, 2000). During embryonic development, brain derived neurotrophic factor (BDNF) plays a major role in neuronal survival and differentiation, as well as promoting survival of neural stem cells isolated from hippocampus postnatally (Shetty and Turner, 1998). Augmented survival of newly generated granule cells in the adult brain induced by manipulations of diet and exercise is associated with increases in BDNF mRNA, and antibodies that block BDNF significantly attenuate the protective effects offered by dietary restriction (Farmer et al., 2004; Mattson, 2000; Lee et al., 2002). Furthermore, increases in BDNF mRNA induced by wheel running in mice are selective for the dentate gyrus of the hippocampal formation and are accompanied by an enhanced capacity for induction and expression of dentate gyrus LTP (Farmer et al., 2004). These findings strongly implicate a role for BDNF in the plasticity in the brain of adult rats. Schneider et al. (2001) reported that animals chronically exposed to Pb display a reduction in mRNA expression of a number of trophic factors, including BDNF, and these effects were partially reversed by environmental enrichment. In a similar experiment, Guilarte et al. (2003) failed to demonstrate a reduction in BDNF
expression by Pb, but did report a housing-induced increase in BDNF mRNA expression in Pb-exposed animals. Although BDNF protein measures were not included in these two studies, the alterations observed in mRNA are suggestive of a role for BDNF in compromised cell survival in hippocampus of Pb-exposed animals.

Considerable debate remains concerning the functional implications of adult neurogenesis. Several laboratories have proposed that survival of newly born granule cells in the adult dentate gyrus is enhanced by learning and LTP (Derrick et al., 2000; Gould et al., 1999; Leuner et al., 2000; Shors et al., 2001) and that neurogenesis may be necessary for some forms of long-term memory (Shors et al., 2002; Snyder et al., 2005). Developmental exposure to Pb induces learning deficits in children (Bellinger et al., 1991; Chiodo et al., 2004), in animal models of cognition (Alber and Strupp, 1996; Cory-Slechta et al., 1995; Hilson and Strupp, 1997), and in physiological measures of synaptic plasticity presumed to underlie learning (Gilbert et al., 1999a; b; Ruan et al., 1998). Results of experiments designed to assess performance of Pb-exposed animals on hippocampal-dependent tasks, however, have been equivocal. In two standard tests of spatial learning, the radial arm maze and the Morris water maze, some studies reported deficits in acquisition (Kuhlmann et al., 1997; Nihei et al., 2000; Zhou and Suszkiw, 2004), while others failed to detect significant impairment (e.g., Driscoll et al., 1998; Guilarte et al., 2002; Jett et al., 1997; Levin et al., 2004; Ma et al., 1999; Munoz et al., 1988; Newman et al., 2002; Schneider et al., 2001; Yang et al., 2003; Elliott and Militec, 1997). Although a number of parameters vary from study to study, disparities are evident even from reports generated from the same laboratory (e.g., Guilarte et al., 2002; Jett et al., 1997; Kuhlmann et al., 1997; Nihei et al., 2000). In the present study, using a standard Morris water
maze procedure, developmental Pb exposure did not impact acquisition of spatial learning. Thus, detecting the subtle alterations in learning associated with developmental Pb exposure may require more complex and demanding cognitive tasks and more sophisticated behavioral analyses (see Alber and Strupp, 1996; Hilson and Strupp, 1997; Cory-Slechta, 1995). The same may be true for identifying the function of hippocampal neurogenesis in learning and memory. Our data are consistent with recent findings reported by Shors et al. (2002) in which neurogenesis in the adult dentate gyrus was found to be essential for some types of hippocampal-dependent learning (i.e., trace fear conditioning), but not spatial learning in the Morris water maze. It is clear that the role that compromised neurogenesis in the adult may play in hippocampal function and its relationship to neurological sequelae of Pb-induced neurotoxicity requires further study.

In summary, we have demonstrated that chronic exposure to low levels of Pb reduces the capacity for structural plasticity in the adult hippocampus. While no impairment in spatial learning was detected, deficiencies in the restorative capacities of the adult brain may contribute to subtle deficits in cognitive ability associated with chronic Pb exposure. The actions of Pb on glutamatergic transmission, neurotrophin-mediated signaling, and synaptic plasticity in the hippocampus may contribute to reductions in the survival of newly generated neurons in the adult dentate gyrus.

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FIGURE LEGENDS

Figure 1. Timeline of Pb and BrdU dosing. Pregnant dams were delivered 0 or 0.2% Pb acetate in drinking water beginning on gestational day (GD)16 until weaning of offspring on postnatal day (PN) 21. Thereafter, 2 males from each Pb exposed litter were administered deionized water, and 2 were continued on the same Pb water as the dam. BrdU was administered twice daily at 12 hour intervals for 12 consecutive days beginning on PN75 (sawtooth marks on Figure). One male per litter from each condition, the control (no Pb exposure), Wean (Pb exposure from GD16-PN21) or Life (Continuous Pb exposure from GD16), was sacrificed 24 hours after the last dose of BrdU, the other litter representative was sacrificed 28 days after the final BrdU injection.

Figure 2. Developmental Pb exposure did not alter the number of BrdU positive cells in the dentate gyrus granule cell layer (GC) 1 day after completion of dosing. Dark pigmented cells represent those staining positively for BrdU. In Control (A, CON), Pb-Wean (B, PW) or Pb-Life (C, PL) exposure conditions. Dark stained profiles are BrdU-stained nuclei of newly born cells. Adult granule cells that were not born in the presence of BrdU appear gray due to nonspecific background staining. The bulk of the BrdU staining cells can be seen lining the inner region of the dentate gyrus granule cell layer (GC) in the subgranular zone (SGZ). BrdU-positive cells in the hilus (H) were not counted. No difference in the number of BrdU-positive profiles per hippocampus was observed among the groups indicating Pb did not interfere with cell proliferation (D). The mean (± SE) number of BrdU-positive profiles within the dentate gyrus cell body layer in different Pb-exposed groups are depicted in D.

Figure 3. Pb exposure reduced the number of BrdU positive cells surviving at 28 days in animals exposed continuously to Pb (C, PL, * Tukey’s, p<0.05). Pb exposure that terminated at weaning appeared to reduce the number of surviving BrdU positive cells (B, PW) relative to controls (A, CON), but this difference was not statistically reliable (D). As in Figure 2, D represents the mean (± SE) number of BrdU-positive profiles within the dentate gyrus cell body layer in different Pb-exposed groups. BrdU-positive cells have moved up from the subgranular zone where they resided at the 24 hour time point (Figure 2). The majority of these cells are now within the granule cell layer itself (arrowheads), where they presumably have been incorporated into the hippocampal network (van Praag et al., 2002). This change in location is clearly evident at higher power in Figure 4.

Figure 4. High power (20X) image of upper blade of dentate gyrus showing surviving BrdU-positive cells in a control (CON, A) and a Pb-exposed (PL, B) animal 28 days after termination of BrdU dosing. BrdU is incorporated into the nuclear material and in granule cells, the bulk of the cell body contains the nucleus. The dark circular appearance of the BrdU-positive cells is similar in shape and location to surrounding paler granule cells. The BrdU-positive cells have migrated from the subgranular zone where they were born (24 hour timepoint in Figure 2) up into the granule cell layer (arrowheads). The morphological profile and the positioning of the cells within the granule cell layer support the conclusion that these BrdU-positive cell profiles represent neurons.
Figure 5. BrdU positive cells surviving at 28 days do not stain for the glial marker, glial fibrillary acidic protein (GFAP). Nissl stained material at increasing magnifications (10X, 40X, 80X) on the left (A, C, D, respectively) demarcates granule cell layer (GC), subgranular zone (S) and the hilus (H). Nissl substance, as BrdU, stains nuclear material such than the distinction in cell phenotype can be estimated by the size of the cell body. Astrocytes (arrows) and granule cells (asterisks) are labeled. Parallel images of tissue double labeled with BrdU and the glial marker GFAP from a control animal are shown in the left hand panel (B, D, E), and are typical of tissue derived from control and Pb-exposed subjects. In both control and Pb-exposed animals, BrdU profiles are dark and round and only the nucleus, which fills the bulk of the cytoplasm of the cell body, stains for BrdU. In contrast, the entire astrocyte is stained by GFAP, smaller brown triangular cell bodies with spiny processes emanating in a radial pattern from the soma (D and E). The astrocytes reside primarily in the hilus, whereas BrdU staining cells can be seen floating above the hilar region within the granule cell layer itself (D) and this is most evident at higher power (E). No evidence of colocalization of GFAP and BrdU was observed. This is indirect evidence that the BrdU is staining newly born granule cell neurons and not glial cells.

Figure 6. Developmental Pb exposure did not alter latency to locate a hidden platform in the Morris water maze. (A) Mean ± SE latency to escape was comparable across groups in 14 days of training. Cue learning with a visible platform was also similar. (B) Probe trials conducted on the first trial on days 3, 6, 9, 12, 15 also failed to reveal differences among groups in the percent of time in a 60 second free swim trial spent in the correct quadrant (Mean ± SE).