Electrophysiologic and Behavioral Effects of Perinatal and Acute Exposure of Rats to Lead and Polychlorinated Biphenyls

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Lead and polychlorinated biphenyls (PCBs) both cause a reduction of intelligence quotient and behavioral abnormalities in exposed children that have features in common with attention deficit hyperactivity disorder. We have used rats as a model to study the effects of both perinatal and acute exposure to lead or PCBs in an effort to compare and understand the mechanisms of these nervous system decrements. Long-term potentiation (LTP) is an electrophysiologic measurement that correlates well with cognitive ability. We have determined the effects of chronic perinatal exposure to lead or PCB 153 as well as acute application of these substances to isolated brain slices, with recordings in two areas of the hippocampus, CA1 and CA3. Both substances, whether chronically or acutely applied, significantly reduced LTP in CA1 in animals at age 30 and 60 days. In CA3, they reduced LTP in 30-day animals but potentiated it in 60-day animals. Although neither lead nor PCB 153 alters baseline synaptic transmission at low stimulus strengths, at higher levels they induce changes in the same direction as those of LTP. These results show surprisingly similar actions of these quite different chemicals, and the similarity of effects on chronic and acute application indicates that effects are both pharmacologic and developmental. Behavioral studies of rats exposed to PCBs from contaminated fish show hyperactivity, impulsiveness, and increased frustration relative to unexposed controls. These results demonstrate that lead and PCBs have similar effects on synaptic plasticity and behavior and suggest that the compounds may act through a common mechanism. *Key words:* **brain slice, extinction, frustration, hippocampus, hyperactivity, input–output curves, long-term potentiation, perinatal exposure.** *Environ Health Perspect* **110(suppl 3):377–386 (2002).**
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One of the most precious, and at the same time one of the most vulnerable, characteristics of human beings is the ability to learn and remember. This is the characteristic that by degree distinguishes humans from animals. Humans vary in their ability to learn and remember, and we measure these abilities in standard tests of intelligence quotient (IQ). Certainly higher animals can learn and remember, and these functions in general increase with relative brain size and complexity. Although the exact mechanisms whereby our brains perform the functions of learning and memory are far from understood, by study of humans performing tasks with modern brain imaging techniques, of humans with brain lesions, of animal behavior, and of the brains of animals, we are beginning to understand some of the brain areas and mechanisms involved.

In humans, Alzheimer's disease is the extreme for loss of these functions, for in Alzheimer's, cognitive ability is lost without, or at least before, interference with many other central nervous system functions such as ability to move, see, and hear. The first symptom is loss of short-term memory. Old memories are ultimately lost as well, but this occurs later in the course of the disease. Although the disease affects many parts of the brain, it is initially localized to the frontal cortex and hippocampus (*1*), areas documented in other studies to have a central role in learning and memory. There is considerable evidence that these two areas mediate different components of learning and memory, with "working memory"—the very short-term memory process involved in such things as remembering a telephone number long enough to dial it—being dependent primarily on the prefrontal cortex (*2*), longer term memory being dependent upon the hippocampus and areas to which it is connected (*3*), while long-term memory, which is initially dependent on the hippocampus, is stored somewhere other than the hippocampus (*4*).

There are other important characteristics of the human personality that are quite different from IQ but at the same time are related to an individual's ability to learn and to be productive and socially interactive. Attention span is one of the most important because it is very difficult to learn without paying attention. A shortened attention span is often expressed as hyperactivity. Another important characteristic is having the ability to deal with frustration and being able to work toward a reward that is delayed, rather than demanding instant gratification. In recent years much more attention has been paid to the syndrome known as attention deficit hyperactivity disorder (ADHD) or attention deficit disorder (ADD). The prevalence of ADHD and ADD is estimated to be from 3 to 7% of children (*5*), and the hyperactivity component is much more common in boys than in girls (*6*). With development, hyperactivity often decreases but not the symptoms of inattention, and at least one-third of children with ADHD still meet the criteria for the disease as adults. About 20% of ADHD children will demonstrate an antisocial personality disorder in adulthood (*7*), and because they have a shortened attention span and an abnormality in the ability to deal with frustration, this disorder may be reflected as sudden violent behavior in adults.

Which areas of the brain control attention span and ability to deal with frustration, and the mechanisms responsible, are even more poorly understood than learning and memory. It is unlikely that the brain areas and mechanisms are identical to learning and memory, although they are certainly related. Although many exceptional people show very high intelligence coupled with impatience and short attention span, children with ADHD (*8*) or prenatal hypothyroidism (*9,10*) usually show the syndrome of minimal brain dysfunction, consisting of somewhat reduced IQ, shortened attention span, and increased antisocial behavior. Similar symptoms have been reported in children prenatally exposed to alcohol and substance abuse (*11*).

Although IQ, as measured by standard tests, is clearly influenced by family and social environment, native intelligence and accompanying personality traits are characteristics that one is born with. In the past these traits have usually been considered to be genetic. However, increasingly we learn that although our genetic make-up certainly puts limits on ability, many factors that are not genetic have important influences on IQ and behavior. That exposure to environmental toxins in the perinatal period can permanently alter IQ,

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behavior, and nervous system functioning was first clearly demonstrated by Needleman and colleagues (*12,13*) for lead. Perinatal exposure to other substances such as methyl mercury (*14*), pesticides (*15*), and polychlorinated biphenyls (PCBs) (*16,17*) causes similar reductions in IQ and disruption of behavior. Adult exposures to lead (*18,19*) and PCBs (*20*) at concentrations that do not cause obvious neuronal cell death have also been reported to reduce cognitive function.

We have used the rat as an animal model to study the actions of two environmental chemicals, lead and PCBs, and have used two methods of study: electrophysiologic recordings from hippocampal brain slices and whole-animal behavior. Other than behavior, one of the best model systems for study of cognitive function is long-term potentiation (LTP), a persistent change in synaptic efficacy that is seen in central synapses in areas known to be involved in higher cerebral functions (*21*). There is considerable evidence that LTP is at least an essential component of the process of learning and memory (*22*). LTP is reduced in animal strains that learn less well (*23*), including mutant animals (*24*), and LTP is also reduced in aged animals (*25,26*). Previous studies in our and other laboratories have shown that LTP is reduced upon exposure to lead (*27,28*) and PCBs (*29*), consistent with our central hypothesis that LTP is a, or perhaps the, central target of environmental agents that alter cognitive function. However, LTP is not pharmacologically the same process in all brain areas. In most of the areas studied to date (piriform cortex, hippocampal area CA1 and dentate gyrus, and visual cortex), LTP depends on activation of the postsynaptic *N*-methyl-D-aspartate (NMDA) form of the glutamate receptor. The NMDA receptor allows calcium ions to enter the cell, leading to a series of biochemical and physiologic responses that ultimately result in long-lasting, and perhaps permanent, increases in the response to a constant stimulus. Other brain areas, including the CA3 area of the hippocampus, exhibit LTP that is similar in outcome but is not related to NMDA receptors on postsynaptic neurons. CA3 LTP appears to depend on presynaptic mechanisms that result in an increased release of transmitter. In the present report we examine the effects of lead and PCBs on these different forms of LTP at different ages. We also report studies on the behavior of rats exposed perinatally to PCB-contaminated fish, where we can measure indicators of impatience, impulsivity, and hyperactivity. Because alteration of these behavioral features appears to parallel effects on IQ in humans, we are attempting to determine whether a similar relationship exists on experimental exposure to lead and PCBs.

Materials and Methods

Animals

All experiments used Wistar or Sprague-Dawley rats (Taconic Farms, Inc., Germantown, NY, USA, or the colony maintained at State University of New York at Cortland). Electrophysiologic experiments were done on animals at either 30 ± 3 days or 60 ± 4 days of age. For *in vivo* exposure to lead, virgin female rats were given 0.1 or 0.2% lead acetate in their drinking water before breeding and throughout gestation and lactation, or during lactation only. After weaning at postnatal day 21 (PD21), some pups were provided lead in drinking water until euthanasia, while others were not further exposed (*30*). The lead concentrations in animals at the two ages with the different exposure paradigms were reported by Hussain et al. (*30*). For *in vivo* PCB exposure, timed-pregnant virgin rats were fed PCB 153 (2,2′,4,4′,5,5′-hexachlorobiphenyl) applied daily on a sweet wafer from gestational day 7 through PD21, at four dose levels (0, 1.25, 5.0, and 20 mg/kg/day) as described by Hussain et al. (*31*). Control studies and studies with acute application of lead or PCB 153 were conducted using brains of animals at the same age that were not exposed to either substance before brain slice preparation. In these studies both lead and PCB 153 were in solution at the limit of their solubility—about 0.5 µM for lead and approximately 3 nM (0.9 ppb) for PCB 153. All studies were conducted with approval by the local animal use committee and with every effort to minimize pain and suffering.

Electrophysiology

Animals were euthanized by cervical dislocation. The brain was quickly removed into ice-cold Krebs-Ringer solution in which all NaCl was replaced by isosmotic sucrose, blocked, and slices of hippocampus were cut at 450 µm on a vibratome, as previously described (*30,31*). The slices were placed in normal, Na^+-containing Krebs-Ringer for at least 1 hr before being mounted in the recording chamber. Stimulating bipolar electrodes were placed on either the Schaffer collateral pathway when recording from CA1 or the mossy fiber pathway when recording from CA3. Stimulation was at a frequency of 0.033 Hz except during tetanic stimulation for induction of LTP, when two 1-sec stimulations at 100 Hz, separated by 5 sec, were applied. The recording electrode, a glass pipette with a resistance of 2–4 MΩ filled with Krebs-Ringer solution, was placed in the dendritic portion of the pyramidal cell layer in either CA1 or CA3. After a stable recording of the population field excitatory postsynaptic potential (fEPSP) was obtained for at least 30 min, the input–output (I–O) relations were determined by varying the stimulus intensity and determining the amplitude of the fEPSP. This was repeated no less than 1 hr after eliciting LTP. Except when measuring the I–O curve, the stimulus strength was kept at 20–30% of maximum. LTP was measured at least 1 hr after application of the tetanic stimulation by determining both the peak fEPSP amplitude and the slope of the rising phase.

Control and *in vivo* exposed rats were treated in identical fashion. In the experiments with *in vitro* exposure to lead or PCB 153, slices were prepared from unexposed animals as described above. For lead studies, a lead-saturated Krebs-Ringer solution (about 0.5 µM) was perfused over the slice after control measurements of the I–O curve and fEPSP. LTP was induced 30 min after beginning perfusion with lead and continued for at least 1 hr. For acute exposure to PCB 153, we prepared a generator column in which distilled water was equilibrated with PCB 153 to the limits of its solubility (reported to be 0.91 ppb) by dissolving PCB 153 in hexane, placing the solution on glass beads, and evaporating the hexane. The distilled water was then slowly circulated over the glass beads containing the PCB 153 for a period of not less than 4 days, and the resulting solution was used to prepare the Krebs-Ringer solution. The PCB-containing Krebs-Ringer solution was perfused over the hippocampal slice for 15 min before tetanic stimulation and for an additional 20 min after induction of LTP, after which time normal Krebs-Ringer solution was perfused. As described above, LTP was measured no less than 1 hr after tetanic stimulation, then the I-O curves were again determined. Other experimental details have been presented in earlier publications (*30,31*).

Behavioral Studies

All methods were as previously described (*32*) except that the studies reported here used animals perinatally exposed to PCBs through ingestion of contaminated fish. Five experimentally naive and primiparous females, maintained on a 12 hr light/dark cycle, were bred using three males. The dams were randomly assigned to a fish food (n = 3) or control (n = 2). During gestation days 9–19, the fish food groups were fed a mash consisting of 27.5 g Purina Laboratory Rodent Diet 5001, 0.95 g corn oil, 0.4 g water, and 1.15 g ground carp caught in Contaminant Cove near Hogansburg, New

York immediately adjacent to the General Motors foundry site on the St. Lawrence River. These fish are contaminated with a mixture of PCBs at a total concentration of approximately 0.72 µg/g. After the exposure period the pregnant rats were placed in separate nesting cages with free access to pellets of uncontaminated Purina Rodent Diet 5001 and water. The offspring were weaned at 21 days, marked for identification, and housed in same-sex pairs. At 65 days of age, 10 animals from each of the four groups—fish-fed males (MFF) and females (FFF) and control males (MC) and females (FC)—were placed on a 22.5-hr water deprivation schedule for subsequent behavioral testing.

The testing was conducted in four BRS-Foringer series 900 and six BRS/LVE model 143 operant chambers in sound-attenuating enclosures (BRS/LVE, Beltsville, MD, USA). The levers required 12 g of dead weight to depress. Response-contingent drops of water were delivered by liquid dippers, accompanied by a cue light. Training sessions were run 6 days a week. One 30-min habituation session was followed by four 30-min dipper-training sessions, during which water was delivered on a variable-time 30-sec schedule. Response shaping sessions were run, followed by five 20-min continuous reinforcement (CRF) sessions to stabilize responding, all with house lights on. All groups were then run through a series of 40-min sessions with a multiple fixed interval, extinction (multiple FI-EXT) schedules of reinforcement. The house light was on during the FI components but off during the 5-min EXT components. Two sessions of multiple 30-sec FI, 5-min EXT were followed by two sessions with multiple 1-min FI, 5-min EXT. The final schedule was multiple 120-sec FI, 5-min EXT. Subsequent sessions were divided into four parts: *a*) a 120-sec FI component in which a maximum of seven reinforcers were delivered, *b*) a 5-min EXT component, *c*) a new 120-sec FI component with the same parameters as the one above, and *d*) a 5-min EXT component that ended the session.

The number of lever presses and reinforcements for each rat were recorded daily. In addition, response bursts, that is, presses with short (≤1.0 sec) interresponse times (IRTs), indicative of hyperactivity (*33*), were recorded during all CRF and FI components of sessions. The 120-sec FI component was divided into four consecutive 30-sec segments, and the 5-min EXT component was divided into five consecutive 1-min segments, during which the numbers of lever presses per segment were recorded. After each operant training session, all animals were returned to their home cages and given water for 45 min.

Results

Electrophysiology

Figure 1 shows LTP in areas CA1 and CA3 of rat hippocampus. Baseline responses to stimulation of the afferent inputs (Schaffer collaterals to CA1, mossy fibers to CA3) elicit an fEPSP that reflects the monosynaptic EPSPs elicited in many different pyramidal neurons in each region. In the isolated, submerged, and perfused brain slice, these responses can be recorded for many hours and are of stable amplitude when the stimulation is at 0.1 Hz. When two 1-sec tetanic stimulations at the same intensity are applied, followed by return to the control frequency, two changes in the response occur. Immediately after the stimulation, the fEPSP shows a large increase that rapidly decays. This is the process known as post-tetanic potentiation (PTP). PTP is seen after tetanic stimulation at many central synapses. But after PTP the response does not go back to baseline, but rather returns to a new and higher level than the control. This higher level is maintained for periods of at least many hours in the isolated slices. This is

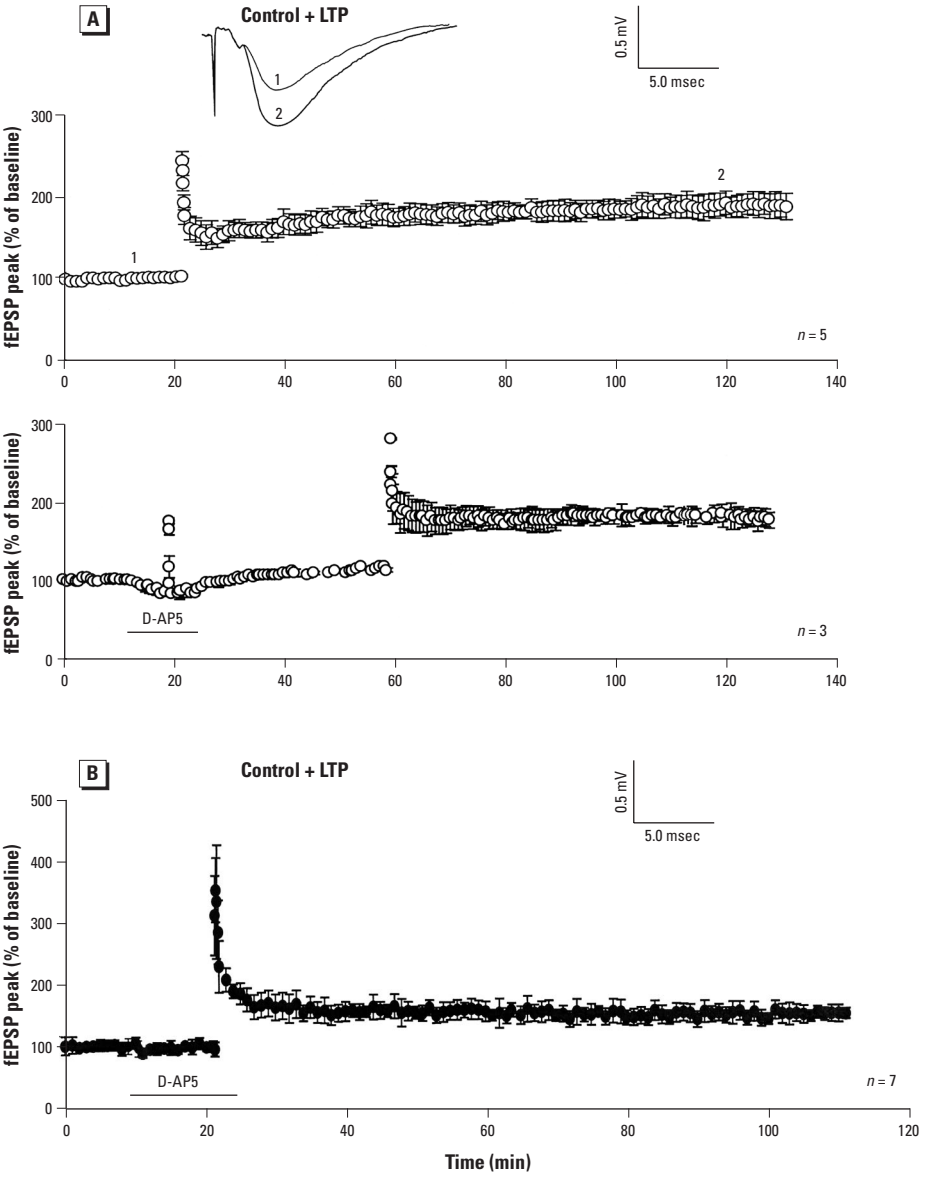

Figure 1. LTP in CA1 (*A*) and CA3 (*B*). The records at the top of both (*A*) and (*B*) show the population fEPSP before (1) and after (2) application of the tetanic stimulation used to induce LTP. The middle record in (*A*) shows LTP, elicited by application of two tetanic stimuli of 1-sec duration, 100 Hz, separated by 5 sec and recorded in normal Krebs-Ringer. The response to stimulation increased to 192 ± 7.5% of control ($n = 5$) at time point *2*, 100 min after initiation. The lower trace shows that stimulation produced no LTP in the presence of 40 µM D-AP5, an antagonist of NMDA receptors. (*B*) shows a similar study in area CA3, initiating LTP in the presence of 40 µM D-AP5. Error bars are ±SEM.

LTP. In Figure 1, *A* shows the fEPSPs and plot of LTP in CA1 and *B* shows similar responses in CA3. Although LTPs in CA1 and CA3 look grossly similar, they have somewhat different pharmacologic sensitivities because the NMDA antagonist D-aminophosphonovaleric acid (D-AP5) blocks LTP in CA1 (*A*, lower trace) but does not do so in CA3 (*B*).

We determined the degree of LTP in rats exposed to lead via the mother's drinking water either through gestation and lactation (to day 21) (perinatal), only by lactation through the mother's drinking water and then in the pup's drinking water until use (post) or from gestation until use (pre and post). Two concentrations of lead were used in the drinking water—0.1 and 0.2%. Figure 2 shows the results obtained in CA1 and CA3 at two ages, 30 days (*A*) and 60 days (*B*). In CA1, LTP is reduced at both ages, and there were no significant differences in the effects of the two lead concentrations or with the duration of exposure. In CA3 there were no differences with time of exposure, but there was a dramatic difference in response as a function of age. At 30 days LTP was significantly reduced, but at 60 days LTP was increased by about 30%.

These results raise the question of whether lead alters only LTP or whether it also affects the synaptic responses in the control. Previously, studies with lead have not reported any effects on the fEPSP with acute application of lead (*27*), and the responses to our usual stimulation intensity in the above studies were not obviously less than those in unexposed animals. However, to study this more systematically, we compared the I-O curves (plotting fEPSP peak amplitude against stimulation strength) in slices from control and lead-exposed animals (Figure 3). In both CA1 and CA3 at 30 days, lead caused a significant reduction in the amplitude of the fEPSP obtained at higher stimulation strengths, although this was not obvious at the stimulus intensity used throughout the experiments (20–30% of maximal response). At 60 days, lead had differential effects on the I-O curves, reducing the response in CA1 but increasing the response in CA3.

We have previously shown that acute perfusion of lead on piriform cortex brain slices reduced LTP. This raises the question of whether the effects of chronic lead exposure seen above reflect a unique sensitivity of the developing nervous system, or whether instead the actions of lead are more pharmacologic and independent of age. To answer this question, we perfused lead acutely over hippocampal slices obtained from unexposed rats at 30 and 60 days of age. Figure 4 shows the results of one experiment from CA3 at 30 and 60 days of age, and Figure 5 shows

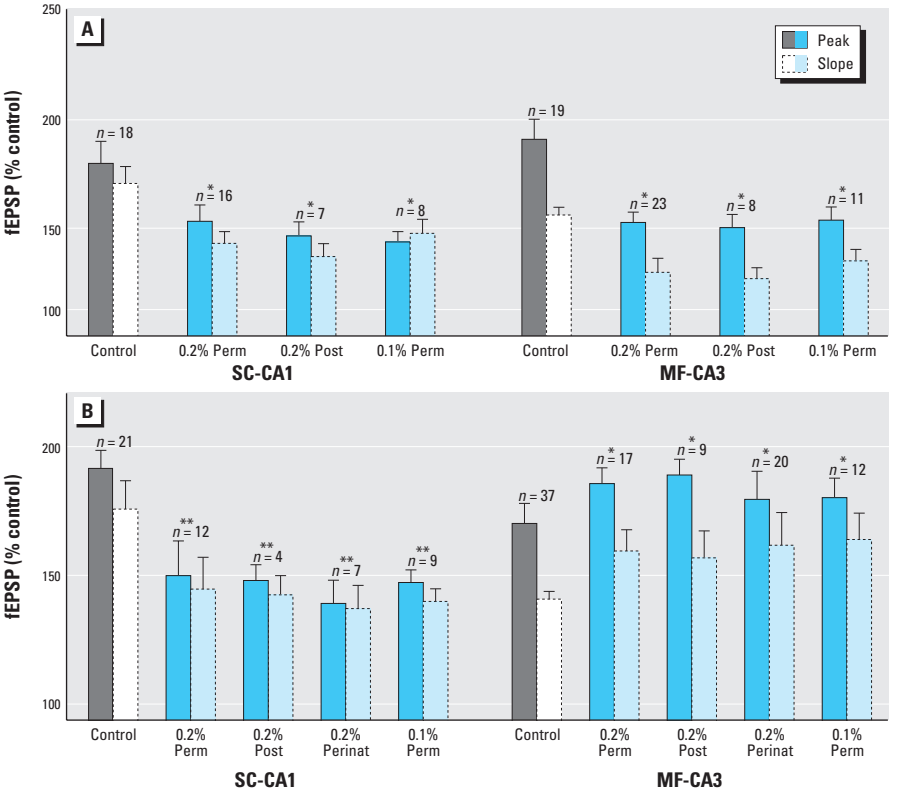

Figure 2. Chronic *in vivo* lead effects on LTP in CA1 and CA3. SC, Schaffer collateral; MF, mossy fiber. The bar graphs show the increase over the control after induction of LTP in fEPSP peak amplitude and slope after various exposures. (*A*) Results from 30-day-old control animals. (*B*) Similar studies of 60-day-old animals. Animals were exposed *a*) to 0.2% lead in the dams' and pups' drinking water from gestation day 3 until sacrifice (0.2% perm), *b*) to 0.2% lead in the dams' and pups' drinking water only after birth (0.2% post), *c*) to 0.2% lead only through the dams' drinking water during gestation and lactation (0.2% perinat), or *d*) to 0.1% lead in the dams' and pups' drinking water from gestation until sacrifice (0.1% perm). Error bars are ±SEM.

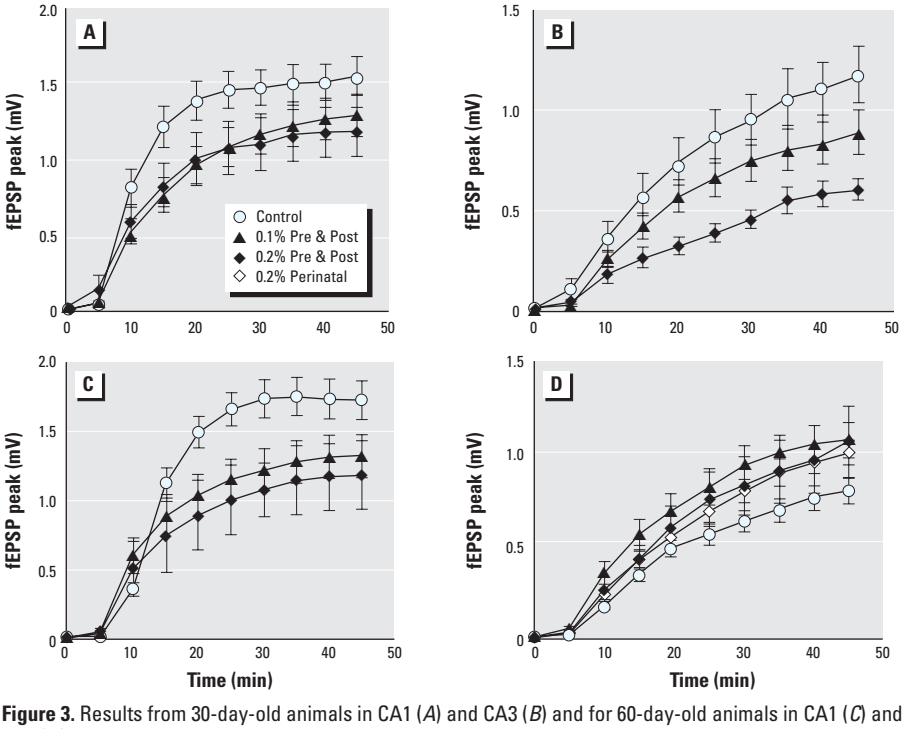

Figure 3. Results from 30-day-old animals in CA1 (*A*) and CA3 (*B*) and for 60-day-old animals in CA1 (*C*) and CA3 (*D*); $n \geq 8$ for all curves.

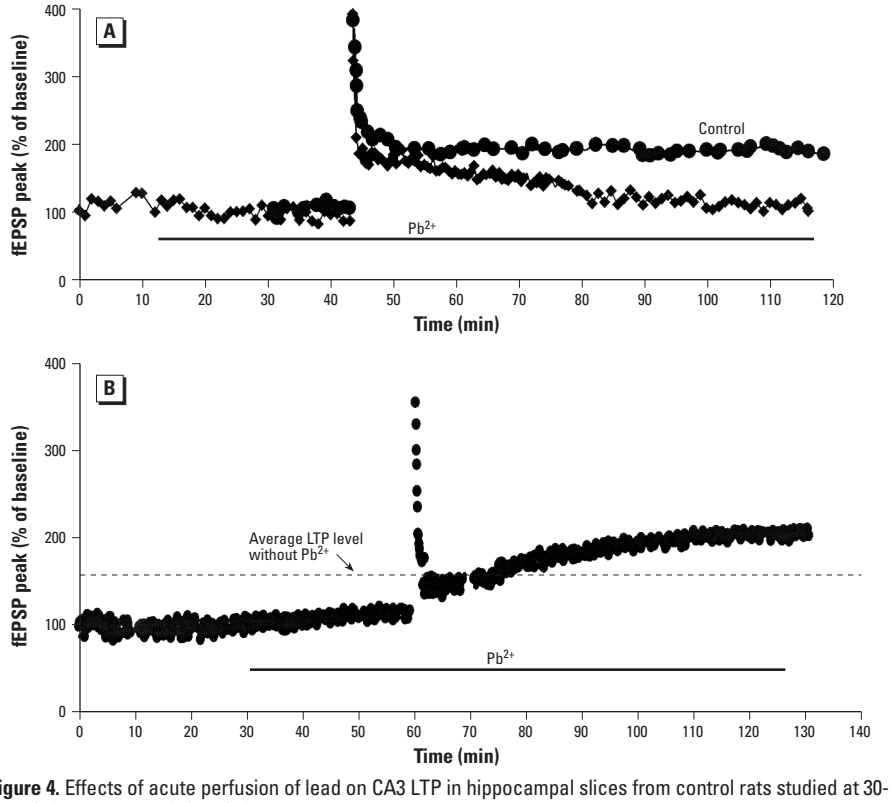

Figure 4. Effects of acute perfusion of lead on CA3 LTP in hippocampal slices from control rats studied at 30-days (*A*) and 60-days (*B*). In (*A*), records from two single experiments are superimposed. The control shows large, maintained LTP. Perfused lead (indicated by the line under the response) produced normal PTP upon tetanic stimulation, but the potentiated response declined back toward control levels. In (*B*), the dashed line indicates the average LTP in CA3 in absence of lead. In the presence of lead, LTP was significantly larger than control.

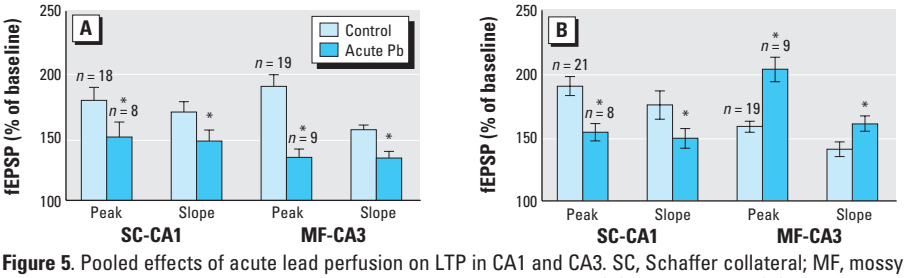

Figure 5. Pooled effects of acute lead perfusion on LTP in CA1 and CA3. SC, Schaffer collateral; MF, mossy fiber. The bars show averages and SEs for fEPSP peak and slope in 30- (*A*) and 60-day-old (*B*) animals.

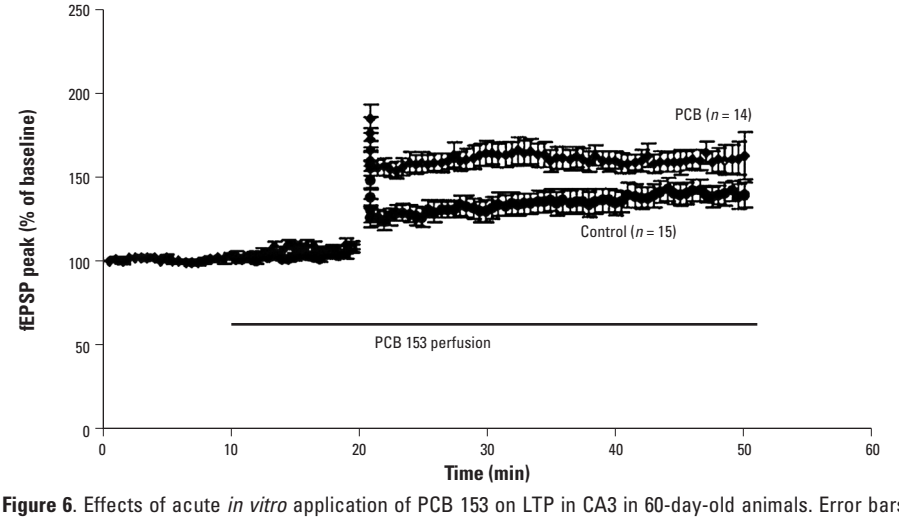

Figure 6. Effects of acute *in vitro* application of PCB 153 on LTP in CA3 in 60-day-old animals. Error bars show SEs. The PCB solution was perfused for 20 min before tetanus.

average changes in both fEPSP peak and slope with from a number of experiments. As in the chronic exposure studies, lead reduced LTP in CA1 at both ages but reduced LTP in CA3 in 30-day animals while potentiating LTP in 60-day animals.

We have previously reported that PCB 153, a di-*ortho*, persistent congener, reduces LTP in CA3 at 30 days of age when animals are exposed perinatally or when acutely perfused over a control slice (*31*). We have not yet measured LTP in CA3 in animals chronically exposed to PCB 153, but we have studied the effects of acute perfusion of this congener in animals at 60 days of age (Figure 6). As with exposure to lead, PCB 153 had differential effects of LTP as a function of age. Although at 30 days of age PCB 153 reduced LTP significantly (*31*), at 60 LTP was increased relative to control. Figure 7 shows I-O curves in the control, after perfusion of PCB 153 before tetanus and after LTP was induced in the presence of PCB 153. Perfusion of PCB 153 has little or no effect at low stimulation strengths but significantly increases the fEPSP amplitude at higher levels. When LTP is induced in the presence of PCB 153, there is a large increase in response at lower stimulus intensities but no significant increase beyond that of PCB 153 alone at higher intensities.

Although the mechanisms responsible for the changing sensitivity of LTP in CA3 are still uncertain, we have evidence, at least for lead, that is consistent with the hypothesis that developmental changes in protein kinase C (PKC) isozyme composition and activity and different actions of lead on different PKC isozymes are responsible (*30,34,35*). Figure 8 shows the effects of perfusion of phorbol ester (an activator of all major PKC isozymes) on synaptic responses from control animals and animals chronically exposed to lead (0.2% lead acetate in drinking water from gestation until use). Phorbol ester causes a significant potentiation of synaptic responses at both ages. However, the magnitude of the potentiation is reduced in animals chronically exposed to lead at 30 days of age

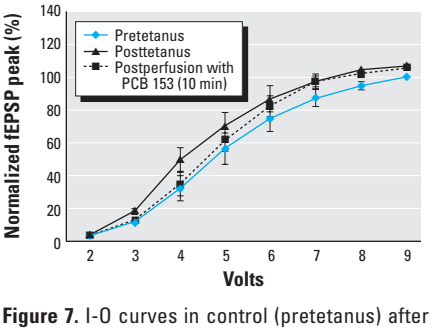

Figure 7. I-O curves in control (pretetanus) after perfusion of PCB 153 for 10 min and at least 60 min after induction of LTP in CA3 from rats at 60 days of age. Error bars are ±SEM.

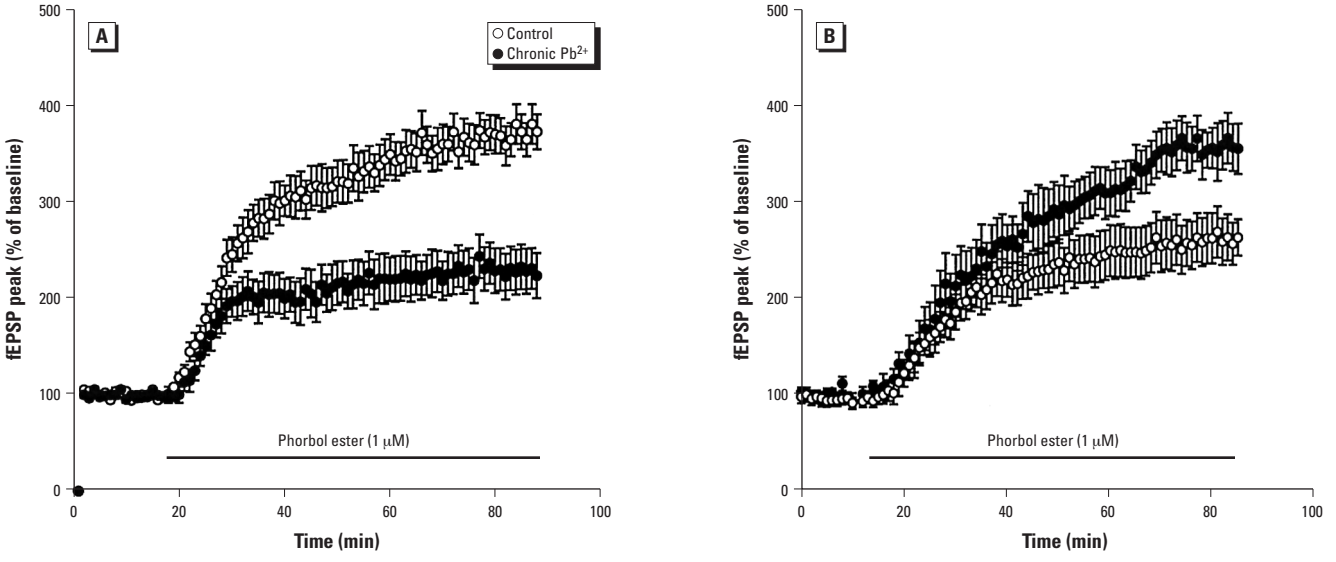

Figure 8. Effects of chronic lead exposure on the potentiation of synaptic responses induced by phorbol ester in area CA3, studied in animals at 30 (*A*) and 60 (*B*) days of age. Note that at 30 days, chronic lead exposure results in a reduction of phorbol ester potentiation, whereas at 60 days it is increased. Error bars are ±SEM.

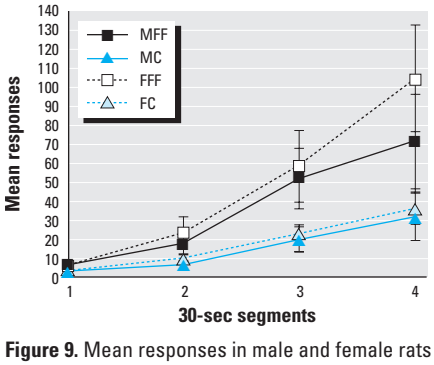

Figure 9. Mean responses in male and female rats exposed to PCBs through consumption of contaminated fish. Error bars are ±SEM.

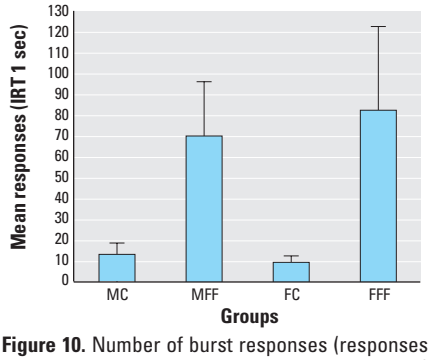

Figure 10. Number of burst responses (responses with an interresponse interval of less than 1 sec) in control and fish-fed male and female rats. Error bars are ±SEM.

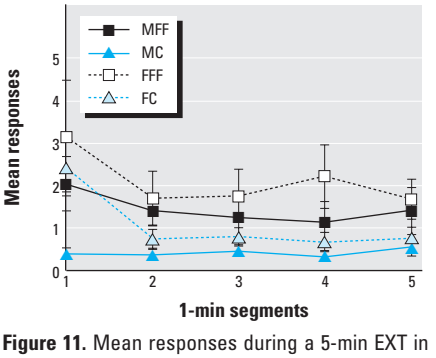

Figure 11. Mean responses during a 5-min EXT in control and fish-fed male and female rats. Error bars are ±SEM.

but increased relative to the controls in animals chronically exposed to lead and studied at 60 days of age. Although this study does not prove that the effects of lead are mediated by alteration of PKC isozymes, the direction of the changes of both LTP and the I-O curves are the same as those on the phorbol ester potentiation. Therefore, PKC isozymes likely are responsible for the changes in the net actions of lead.

Behavioral

The next question of importance is whether exposure to PCBs and lead affects behavior in this animal model. Others have reported decrements in both learning and memory functions in animals exposed to either lead (*36*) or PCBs (*37*). Lead-exposed animals demonstrate changes in behavior as well. Rice (*38*) has reported that monkeys exposed to lead show increased distractibility, inability to inhibit inappropriate responding, and perseveration in behaviors no longer appropriate, whereas Alber and Strupp (*39*) interpret the effects of lead exposure in rats as reflecting impatience when delays are longer. We have studied behavior of rats exposed to either PCBs mixed into food (*32*) or rats fed fish that have significant PCB contamination. In our published study, adolescent (30-day-old) male rats fed either contaminated fish or PCB-contaminated rat chow for 30 days showed hyperactivity and impulsiveness. We have now expanded these studies to include female and male rats exposed perinatally by feeding the dam rat chow containing PCB-contaminated fish for gestation days 9–19. The exposure of the pups was both during gestation and via lactation.

Figure 9 shows tests of hyperactivity and impulsiveness. Hyperactivity is indicated by the tendency of exposed animals to press the lever more often and to produce more response bursts (Figure 10) than unexposed control animals during the FI component of the multiple schedule. Impulsiveness is responding prematurely in anticipation of the end the fixed time interval before the next reinforcer. Figure 9 shows the group means of each animal's mean number of lever presses over the last five sessions (stable state) plotted over 30-sec segments of the 120-sec FI. The hyperactivity of the fish-fed groups, compared with control groups, was particularly pronounced by 90 and 120 sec of the FI. A 4 × 4 mixed analysis of variance (ANOVA; groups × consecutive 30-sec segments of FI) showed a significant main effect segment [$F(3,108) = 30.59$, $p < 0.0001$] and a significant groups × segments interaction [$F(9,108) = 2.61$, $p = 0.0089$]. Newman-Keuls comparisons of the interaction means revealed that the FFF group was significantly different from both control groups (FC and MC) at 120-sec (all p values < 0.001) and also from the MFF group (all p values < 0.01). The MFF group also responded more at 120 sec than both control groups (all p values < 0.03).

Figure 10 presents the groups' mean numbers of response bursts during stable state. The exposed offspring tended to produce more response bursts than control offspring, but the data were quite variable (mean square error = 5883). A one-way

ANOVA was marginally significant [$F(3, 36) = 2.40$, $p = 0.08$].

Figure 11 shows responses during a 5-min EXT. Even though this responding was at a lower rate compared with that during FI, indicating that discrimination learning had occurred, the exposed animals, both male and female, showed higher rates of responding than controls. We used the mean numbers of lever presses during stable state to compute a 4 × 5 mixed ANOVA (groups × consecutive 1-min segments of EXT) as above. The analysis yielded only significant main effects of groups [$F(3,36) = 4.33$, $p = 0.01$] and minutes [$F(4,144) = 3.81$, $p = 0.006$] and no interaction. Newman-Keuls comparisons revealed that the differences between the exposed groups and their same-sex controls (MFF vs. MC, and FFF vs. FC) were not significant ($p = 0.094$ and $p = 0.096$, respectively). These tests also showed that the animals pressed more during minute 1 than during minutes 2–5 (all p values < 0.02); minutes 2–5 did not differ.

Discussion

These results provide several new insights into the actions of lead and PCBs on neuronal function in a rat model of the neurobehavioral effects of these contaminants. The major new conclusions are as follows:

a) Lead and PCBs have remarkably similar actions on LTP, including a change from reducing to facilitating LTP in hippocampal area CA3 with development. This observation suggests the possibility that they have similar, but at present uncertain, mechanisms of action.

b) For both lead and PCBs, chronic developmental exposure to the contaminant has similar effects as acute administration to brain slices from normal animals. Although this observation does not necessarily rule out irreversible developmental effects, it does indicate that at least a component the neuronal function decrement is more pharmacologic.

c) Lead, and possibly also PCBs, does alter the baseline synaptic responsiveness in hippocampal CA1 and CA3, contrary to previous conclusions from our and others' laboratories. Although these actions are apparent only at relatively high stimulus strengths and have been demonstrated only in two areas of the hippocampus, they suggest that lead and PCBs interfere with some fundamental process involved in synaptic transmission as well as synaptic plasticity.

d) Although LTP has been correlated with learning ability in many animal studies, the relationship between effects on LTP and behavioral effects related to hyperactivity, frustration, and impulsiveness is unclear.

e) Although LTP is clearly a valuable model for study of cognitive ability, it is not a single process, and it has different developmental and pharmacologic characteristics in different brain areas. Therefore, extrapolating from animal studies of LTP to human neurobehavioral effects of environmental agents must be done with caution.

Lead and PCBs Have Similar Effects on LTP

Lead and PCBs have very similar actions in humans and in animals. In humans, lead exposure in the first few years of life, depending on the level of exposure, may result in a reduction of IQ of up to about 5–7 IQ points and a number of behavioral abnormalities that reflect a shortened attention span, hyperactivity, and a greater tendency for antisocial behaviors (*12*). PCBs also cause a similar reduction in IQ (*16,17*) and have been shown to induce similar behavioral effects in some human studies (*40*) and in animal studies (*41–43*).

We (*27,29–31*) and others (*28,44,45*) have shown that LTP is reduced by both lead and PCBs in certain brain areas, including the piriform cortex, hippocampal CA1 and dentate gyrus, and visual cortex. Hippocampal CA3 has been less studied, although Gutowski et al. (*46*) report that they did not observe any effect of developmental exposure to lead on LTP at this synapse. Their study and ours have several technical differences, and the different results may reflect either these differences or the ages of the animals at the time of the experiments. Clearly, however, neither lead nor PCBs affect all forms of LTP in identical manners. These differences are probably more complex than simply NMDA-dependent versus NMDA-independent forms of LTP because Altmann et al. (*44*) report differences between visual cortex and hippocampus. This conclusion obviously complicates relating LTP to learning and behavior because now the question becomes not just what these xenobiotics do to LTP but also what they do to LTP in which brain region and at which age. Unfortunately, the brain regions that play the most critical roles in any of these neuronal functions are not known.

The parallels between the effects of lead and PCBs in terms of human responses as well as actions on LTP (most striking regarding developmental patterns in CA3) suggest a common mechanism. This is somewhat surprising, because these are very different chemicals, but might reflect actions on the same end point without necessarily requiring identical chemical reactions. Both lead and PCBs have multiple actions in biological systems, and we do not know which, if any, of these known actions is responsible for the learning and neurobehavioral effects. Our working hypothesis is that LTP is the process that is altered by both acute and chronic exposures to lead and PCBs, and that the common site of action of these two different chemicals is PKC. PKC activation is essential to LTP in both CA1 and CA3 (*47,48*). PKC activity is altered by lead in a complex fashion, both activating the enzyme and inhibiting it by competitive and noncompetitive actions (*49*). The various PKC isozymes are affected differentially (*50*). PKC has at least 11 different isozymes, each with differences in cofactor dependence and response to phospholipid metabolites (*51*). Hussain (*34*), using PKC antagonists specific for different isozymes, has shown developmental changes and differential distribution of PKC subtypes in CA1 and CA3. Study of these effects is still in progress, but our hypothesis is that the differential effects of lead in CA3 will be explained by differential actions of lead on the PKC subtypes. The effects of PCBs on PKC have been much less studied, although Kodavanti et al. (*52*) have reported that exposure to Aroclor 1254 resulted in a decrease in total cerebellar PKC activity but an elevation of membrane-bound PKC activity.

Lead and PCBs Both Have Similar Effects with Acute and Chronic Exposures

It has been generally accepted that the developing nervous system has a unique vulnerability to both lead and PCBs. Needleman et al. (*13*) found that children exposed to lead during development still had cognitive and neurobehavioral decrements years later, and similar results for PCBs were reported by Chen et al. (*53*). Although these observations are convincing, the conclusion that the adult nervous system is no longer sensitive to low concentrations of lead or PCBs may not be true. Muldoon et al. (*18*) found that elderly women with blood lead levels ≥8 μg/L performed more poorly on a cognitive test than women with blood lead levels ≤3 μg/dL. Cognitive decrements have been repeatedly shown after occupational exposure to lead (*19*). Schantz et al. (*20*) have found that adults who ate significant amounts of contaminated fish from Lake Michigan have deficits of learning and memory that are significantly related only to PCBs in the fish, and not to 10 other contaminants examined, including lead and methyl mercury. These observations indicate that lead and PCBs affect the process underlying cognitive function and behavior in adults as well as during development.

In our experiments, we found very similar effects on LTP in both CA1 and CA3 when animals were exposed perinatally and when the chemical was perfused onto a previously unexposed brain slice. The surprising but obvious conclusion from this observation is that the action of lead and PCBs is more of a pharmacologic effect than a disruption of development. This is not to deny a developmental effect of either substance in humans, although our studies do not demonstrate unique developmental action in our animal model. The recent study of Rogan et al. (*54*) shows without question that lead exposure in children results in permanent loss of cognitive potential that is not reversed even if the chelator succimer is used to lower serum lead levels. However, both lead and PCBs are persistent substances, with reservoirs in bones and adipose tissue, respectively, so in the usual situation exposure is prolonged. It is not clear why we did not see a specific developmental effect in our studies. It may be that the rat is not an adequate model for human neurobehavior. Nevertheless, our results clearly indicate that lead and PCBs have acute effects on adult brain that may explain the cognitive decrements seen in adults exposed to these substances.

Lead and PCBs Alter Synaptic Responses in Addition to LTP

Previous studies in our (*27,29*) and others' (*44,45*) laboratories have not revealed effects of either lead or PCBs on baseline synaptic responses, although all of these studies reported an action on LTP that was presumed to be specific. Therefore, we were somewhat surprised that when we obtained full I-O relations with both lead and PCBs, we found a reduction in response at higher stimulus strengths. This indicates an effect of both substances on basal synaptic transmission, and that the actions are not specific to plastic events only. Most previous studies were made at a stimulus strength that gave a response that was much less than maximal, so that the increase with LTP would be apparent. In our studies we routinely obtain the I-O relations, then proceed with a stimulus strength that is about 20% of maximal, and this stimulus strength produced no significant effect before induction of LTP. The report by Gilbert et al. (*45*) is the only study to our knowledge in which effects of PCBs on I-O relationships were studied, and they did not observe any such effects in the dentate gyrus.

The mechanisms responsible for these actions are not clear but again are a clue for further investigation of the sites of action for both substances. Because the direction of effects on the I-O curves with development were the same as that of the effects on LTP, it seems likely that the same mechanism is involved. This also could be an effect on PKC because PKC activators such as phorbol ester cause changes in the baseline synaptic responses (*48*).

Neurobehavioral Effects of Lead and PCBs and Their Relationship to LTP

The cerebral localization of such emotions as impatience, hyperactivity, and frustration is not known with any certainty, although they are usually presumed to be frontal cortical traits. They are usually assumed to be quite independent from memory and cognitive ability, functions that depend strongly on the hippocampus and its connections.

The data from our studies support the hypothesis that the exposed groups would become hyperactive and impulsive. Their overactivity was shown by the tendency, at stable state, to press the lever more often than unexposed control animals during the FI component of the multiple FI-EXT schedule. The fact that these increases in pressing occurred primarily during the 90-sec segment, and reached statistical significance at the 120-sec segment, of the 2-min FI periods shows the animals' impulsiveness because they respond prematurely in anticipation of the end of the time interval before the next reinforcer. The exposed animals also produced relatively more response bursts than the control animals, which is another component of hyperactive behavior. What we did not expect was the significant difference between the exposed male and female animals at the 120-sec segment of the FI.

The first study to use the two-component multiple 2-min FI, 5-min EXT schedule of reinforcement with female rats (*55*) failed to find hyperactivity or impulsiveness in animals exposed to PCBs. They dosed nursing dams via gavage with 5 mg/kg body weight of PCB 153. The pups were therefore exposed through the dams' milk throughout nursing. Exposed females were not different from controls on measures of number of lever presses within the FI (the measure of impulsiveness) or IRTs (bursting), a measure of hyperactivity. Schantz et al. (*56*) perinatally exposed male and female rats to one of three *ortho*-substituted PCBs (28, 118, or 153) and tested the animals on a delayed spatial-alternation task. At the higher doses of all PCB congeners and for all periods of delay, significantly slower acquisition (relative to controls) was found in female but not in male rats. Thus, only lactational exposure (*55*) did not affect the performance of female rats, but perinatal exposure (*56*) led to sex differences, with exposure to PCBs affecting females and not males. Although the task employed by Schantz et al. (*56*) (a measure of learning or memory) is very different from the task employed in the present study (operant FI-EXT), the combined results of our study with contaminated fish and the Schantz findings with PCB congeners suggest that the effect of perinatal exposure is dependent upon the sex of the animal. This conclusion is also supported by the results of Geller et al. (*57*), who administered Aroclor 1254 to pregnant dams by oral gavage from gestational day 6 through PD21. Male and female offspring were then tested in a signal detection task capable of assessing sensory thresholds. Training included autoshaping and operant conditioning. They found autoshaping to be generally faster in males than in females in both exposed animals and controls. However, the speed of autoshaping was reversed by exposure to Aroclor 1254. That is, perinatal exposure retarded the onset of lever pressing for the males but accelerated the onset for females. Performance of male rats exposed to 6.0 mg/kg of Aroclor 1254 was close to that of control females, and the performance of females exposed to the same dose was close to that of control males. Similar effects were found for behavioral assessment of visual thresholds. Control females needed less light to perform at criterion under absolute and increment threshold conditions. However, exposure to Aroclor 1254 made the male rats more sensitive, that is, more like females. Geller et al. (*57*) state, "There was a trend toward the dosed females being less sensitive i.e., more like males," and ". . . the major effects noted were gender-specific alterations in two sexually dimorphic tasks, autoshaping and psychophysical luminance threshold determination" (p. 274). They suggest that their findings for male animals were a result of the antiandrogenic effects of Aroclor 1254, and those for female animals, the ability of Aroclor 1254 to interfere with the estrogenic system. Widholm et al. (*58*) have also reported sex-specific effects of PCBs on associative ability and inhibitory control.

The link between PCBs and hyperactivity may be related to the ability of *ortho*-substituted PCBs to alter dopamine levels. *In vitro* studies (*59,60*) have demonstrated reduced levels of cellular dopamine in PC12 cells that were exposed to PCBs, presumably via inhibition of tyrosine hydroxylase. *In vivo* studies with monkeys (*59,61*) and rats (*62*) also found reduced levels of dopamine in the brains of exposed animals. However, Zahalka et al. (*63*) were not able to replicate these observations in a different strain of rats. Seegal et al. (*62*) measured dopamine levels in the frontal cortex, nucleus accumbens, caudate nucleus, hippocampus, and substantia nigra of rats fed

diets supplemented with contaminated (either 5 or 20% w/w) lyophilized fillets of salmon from Lake Huron or Lake Ontario. Significant reductions in dopamine were found in the frontal cortex and caudate nucleus. Seegal et al. suggested that the reduction in biogenic amines from contaminants in the fish may affect behaviors that require inhibition of previously rewarded responses. The PCBs in the contaminated fish (the congener profile of the fish resembled a mixture of Aroclors 1254 and 1260) may therefore have reduced dopamine levels in the exposed animals, and the reduction of dopamine precluded the animals' abilities to inhibit responding early in the FI. PCBs also have other possible sites of action. For example, PCBs are known to inhibit gap junctions, which can inhibit cell-to-cell communication (*64*), but the role of these actions has not been further studied.

Is LTP a Good Model System for Study of Nervous System Actions of Lead and PCBs?

An excellent recent review (*22*) of the relationship between synaptic plasticity and memory argues strongly that LTP is an excellent model system for study of memory mechanisms but also cautions that although LTP appears to be an essential component of memory, it is by no means everything. In essence, LTP is necessary but not sufficient to explain memory. But the larger question of the relationships between memory and behavioral responses, such as hyperactivity and shortened attention span, is even more uncertain, because the brain organization and mechanisms behind these behavioral traits are even less understood than memory and cognition. PCBs and lead have indisputable direct effects on the nervous system, as demonstrated in studies in which the exposure is to isolated brain slices. However, the *in vivo* preparation has the potential to show interactions with many other parameters, such as the degree of alternation of the dopamine system and activities of various hormones, including estrogens, androgens, and thyroid hormone. There is great promise in studies that couple LTP measurements with direct behavioral observations on the same animals, or at least animals similarly exposed, and especially if levels of kinases, neurotransmitters, and hormones are also determined. Our observations, at a minimum, provide hypotheses for future studies on the mechanisms by which lead and PCBs interfere with cognitive function, and support a strong relationship between cognition and behaviors reflecting activity, impulsiveness, and ability to deal with frustration.

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