Behavioral Effects of Early Postnatal Lead Exposure in Herring Gull *(Larus argentatus)* **Chicks**

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BURGER, J. *Behavioral effects of early postnatal lead exposure in herring gull* (Larus argentatus) *chicks.* PHARMACOL BIOCHEM BEHAV 35(1) 7-13, 1990.—Lead exposure early in life affects behavioral and intellectual development in humans. In this paper, I use the herring gull, *Larus argentatus,* as an animal model to examine effects of lead exposure on early development. Like humans, birds rely mainly on visual and vocal, rather than olfactory, modes of communication. Each of 24 one-day-old herring gull chicks was randomly assigned to one of three treatment groups to receive a lead nitrate solution at a concentration of 0.0, 0.1 and 0.2 mg/g. The control dose was an equal volume of sterile saline. The trios were not siblings, but were matched by weight. Behavioral tests were performed either daily, every two to five days, or at the end of the experiment (45 days posthatching), depending on the nature of the experiment. The behavioral tests examined locomotion, balance, righting response, begging, recognition, thermoregulation and visual cliff. Although on most days, begging behavior, balance and righting response did not differ significantly, over the 45 days of the experiment control birds performed better on more days than the lead-injected birds. Balance was disturbed by lead-injection for the first six days following injection. Individual recognition developed by day 5 in control birds, by day 10 for 0.1 Pb mg/g birds, and by day 14 for 0.2 Pb mg/g birds. Depth perception and thermoregulation behavior were also adversely affected by lead.

Lead Postnatal Behavioral Gulls

PRENATAL and childhood exposure to metals in humans and other primates may lead to retarded psychomotor development (1, 7, 8, 20, 21, 40, 46, 56). Similarly, heavy metals influence such diverse behavior as burrowing in invertebrates, *Corophium volutator* (26), avoidance in grass shrimp, *Palaemonetes pugio* (3), and *Coturnix* quail (44) and reproduction in mallards, *Anas platyrhynchos* (35). Despite the general decrease in lead in the environment, and the overall decrease in lead in children in recent years, there has been an increase in childhood lead in the United States (63), thus, it is important to determine effects of low-level lead exposure in animals as models.

Birds are useful models for metal toxicity because they share with humans a reliance on visual and vocal communication, in contrast to the olfactory and ultrasonic modes of information transfer in rodents. Most birds have a neonatal developmental period when they are dependent on their parents for provisioning of food and protection form predators. In this paper, I report on the effects of lead exposure on behavior of herring gulls, *Larus argentatus,* exposed only once at two days of age. I examine the effects of lead on balance, locomotion and righting, begging, recognition, thermoregulation, and depth perception, behaviors relevant to survival in nature. One advantage of working with gulls is that the experimental paradigms relate directly to natural behaviors and adaptations, and the behavior of gulls in the wild has been extensively studied (25, 30, 34, 38, 51, 52, 53).

Early studies on heavy metals documented tissue concentrations, metal uptake and fates, and concentrated on examining the lethal effects of metals. More recently, authors have concentrated on sublethal, physiological, developmental, and behavioral effects. Lead affects schedule-controlled behavior and performance in pigeons, *Columbia livia* (4, 24, 39) and physiological and anatomical parameters (38); as well as reproductive behavior in ringed doves, *Streptopelia risoria* (43). However, in other species, increased lead levels in naturally occurring populations does not decrease reproductive success (31). Neurobehavioral ontogeny, neurochemistry, and neurotransmitters are also adversely affected by lead (6, 29, 37). Many of these experiments involved laboratory paradigms not directly related to naturally occurring behavior and survival, or involved observations of a broad nature where specific behaviors could not be related to particular lead exposures. In this study, I controlled the timing and level of exposure, and test herring gulls with paradigms directly related to naturally occurring behavior.

Herring gulls are ideal for these experiments because they are large, easy to raise in the laboratory, adapt readily to human handling, eat a variety of readily obtained foods, and there is a voluminous literature concerning field and laboratory experiments with them (5, 9, 41, 42, 51, 54, 60, 64). Moreover, their natural populations are large and growing, so that they are considered a pest species to be controlled near airports and near breeding colonies of other, smaller, more vulnerable species. Finally, I have made behavioral observations on herring gulls in nature over the last ten years (10-15). Some of these experiments were conducted with terns (16), but the experiments were less extensive and did not examine temporal differences. Further, tern populations are vulnerable and it is necessary to develope an expendable avian model.

METHOD

Under appropriate federal and state permits, I collected 24 one-day-old herring gull chicks from a salt marsh colony in Barnegat Bay, NJ in 1987. Only the first hatched chick in any nest was collected to eliminate possible biases due to hatch order; thus, none of the chicks were siblings. Chicks were marked with numbered leg-bands for identification. Chicks were matched for weight, and randomly allocated to one of three treatment groups.

Chicks were housed in groups of three (one from each treatment) in cages, and maintained in a wanned laboratory at 27 ± 2 °C with a natural light-dark cycle. Four times daily they were fed a diet of whole fish and squid similar to their natural diet. Chicks were maintained until 45 days of age when they were sacrificed.

Exposure

At 2 days of age chicks were given a single intraperitoneal injection of lead nitrate (0.1 or 0.2 mg/g of lead in 50 mg/ml sterile water) or sterile saline solution. Control chicks were injected in the same manner as experimental chicks. Lead injection was performed by a laboratory technician who was otherwise not involved in the experiment, and the identity of exposure levels was not revealed to persons performing the behavioral tests. I used a single dose to eliminate conflicting results due to dose, and the lead was injected at 2 days to insure early exposure on chicks that are clearly able to stand and explore. When chicks are allowed to eat food laced with lead they eat different amounts and do not obtain the same dose.

Testing

Some tests were performed daily (balance, righting response, begging), others were performed every 3-5 days (individual recognition, thermoregulation, visual cliff), and others were performed at the end of the experiment (cliff, incline, swallowing). This combination of tests was used to evaluate balance and locomotion, normal begging, depth perception, thermoregulation and individual recognition. Tests that might involve habitation were performed less often.

Before the first feeding each day begging was solicited by holding the feeding forceps in front of the chick, and noting the intensity of *begging,* scored from 0 (no begging) to 10 [vociferous begging accompanied by jumping up and down and flapping wings, see Burger and Gochfeld (16)]. Prior to feeding, *righting* response was measured by putting the chick on its back and recording the time it required to right itself to a standing position. The chick was then placed on a narrow board (4'cm wide and 35 cm long) and allowed to walk to test *balance* and distance walked. Balance was scored on a scale of 0 (fell off immediately) to 5 (remained upright without using any body movements for balance). Then chicks were weighed and fed.

Recognition was tested by comparing their response to the person who fed them (caretaker) with their response to a stranger. The same person fed the chicks each day, and recognition was tested by having the female caretaker on one side of the table, and a similarly sized technician (with the same color and length of hair) on the other side, 60 cm away. The location of the caretaker was switched before every new series of tests from one day to the next to prevent the chicks from learning the position of the caretaker. Each person held a similarly sized fish in a forceps at an

FIG. 1. Thermoregulation (top) and visual cliff (bottom) apparatus. The sun provides shade in one location without a vertical object, while the vertical object provides no shade. On the visual cliff the chick can step over the interior cliff edge (A), onto the clear surface, or can fall over the exterior cliff edge (B). Chicks on both tests were placed in the center.

equal height above the table. A chick deprived of food for 3 hr was placed under an opaque cup on the center facing perpendicularly to the two persons and was allowed to acclimate for 1 min. When the cup was removed, I recorded the time to respond, the number of body turns before going to one of the technicians, which person was approached (caretaker vs. other) and the distance moved before eating any food (if it did so).

Thermoregulation was examined by placing a chick in the center of an apparatus (Fig. 1) that offered choices between full sun and temperatures over 37° , a raised object that provided no shade, or a shaded area without a raised object. The test ran for 2 min, and the substrate temperature was 27-29°C in the shade and 37-43°C in full sun. I recorded the time for the chick to reach the shade, the total time in the shade, and the total number of calls given by the test chick. In nature, nests are often in full sun, and chicks must seek shade as the day becomes hotter. Depth perception was tested on a *visual cliff* (Fig. 1) where the chicks could move about on a solid opaque surface, cross onto a transparent surface, or jump or fall off the sides. The apparatus was 40 cm high. I recorded a score for their performance, and the total number of peerings given at the cliff edge. Peering is when the chick stops abruptly at the cliff edge, with its feet at the edge, its body well back from the edge, and with its head peering over the edge. The chick's behavior was scored as: $0 =$ chick didn't move, $2 =$ chick moved onto interior clear surface (fell over interior cliff), 3 = chick fell off outer cliff, 4 = chick moved onto and remained on interior opaque (safe) surface, 5 = chick moved onto outer opaque surface, 6 = chick went to actual cliff edge but did not fall or peer, and $7-10$ = chick went to cliff edge and performed $1-15$ peerings at the edge.

FIG. 2. Responses of all herring gull chicks to the choice of a caretaker who has been feeding them and a similarly appearing person (bottom). Percentage of correct responses (to their caretaker) is a function of lead-treatment (top).

Because the visual cliff was not very high, at the conclusion of the experiment (45 days of age), I tested the chicks on a 2 m high cliff, holding a fish in a forceps 8 cm from the edge of the cliff. I recorded the distance the chick stayed back from the edge, and scored their behavior: $0 =$ moved back from the edge, $2 =$ stayed where they were placed, $4 =$ moved toward edge, but stayed at least 25 cm away, $6 =$ moved toward cliff, but stayed at least 20 cm from edge, $8 =$ moved toward cliff, but stayed 10 cm from edge, and $10 =$ moved to edge of cliff with feet at edge, reached over edge to successfully secure fish while maintaining balance.

I tested *balance* at 45 days of age by placing chicks on a horizontal board elevated at a 25° angle from the horizontal. The board was covered with sand paper to provide traction. The incline was then slowly raised to see the maximum angle where they could maintain balance and remain on the board. I also recorded the distance they could move on the incline. At 45 days I examined feeding ability by measuring the time required to swallow a fish presented in a forceps. Fish were of equal size and chicks were tested with two fish in rapid succession.

Since the chicks were acclimated to people, they showed no

signs of fear or escape behavior during any of the tests. Visual cliff, thermoregulation, incline, and fish-swallowing tests were performed following feeding so the chicks were satiated and did not beg from the technician. During all tests chicks were in visual and vocal isolation from the other chicks, wherever possible the technicians were also hidden from view.

Statistical Tests

I used multiple regression procedures to determine if treatment and age contributed to differences in behavioral responses (2). For every behavioral test both factors entered as significant variables $(p<0.05)$. Thus, I used the following tests to distinguish between treatments.

I tested behavioral differences using Kruskal-Wallis χ^2 tests (58). To avoid violating assumptions of the test, Kruskal-Wallis χ^2 tests were performed for each day. However, with only 8 birds per group, and with variations in behavior, subtle differences may not show up that are nonetheless present. In addition to examining daily differences, I also examined the overall pattern of performance by assuming that if there are no differences as a function of treatment, then each group should perform best on one third of the test days. If scores were equal, then that day was eliminated from the sample. This test used the mean daily scores for each group on each test; analyzed with a chi-square Goodness of Fit test (59). Any significant deviation from this pattern is shown in Table 1.

RESULTS

By 8 days postinjection, the control birds were heavier than the lead-injected birds, and other body measurements also differed (18). By 45 days of age, the control birds were still heavier, but the lead-injected bird had attained similar bill lengths.

Begging Behavior

Begging is an obvious behavior in young gulls, and it was easily conditioned to be directed at the investigator by holding an empty forceps towards the young gull. Begging behavior following injection did not differ significantly as a function of treatment for any day (Table 1). However, in comparing the relative values over the entire experiment, control gulls showed higher begging intensity on significantly more days than did lead-injected gulls (Table 1).

Balance

Balance was measured on a 4 cm wide board. The balance of lead-injected birds was affected on days 3-8 when they performed less well every day (Table 1). For the duration of the experiment, however, the control birds performed significantly better on only one day, but they performed better on significantly more days than lead-treated birds. Although the distance walked on any day did not differ significantly as a function of treatment, the control birds did perform better on more days than the lead-injected birds (Table 1).

The time required to right itself was significantly longer on the day following injection for the lead-injected birds compared to the controls, but not on any other day thereafter (Table 1). However, when considered cumulatively, control chicks had significantly more days with quicker righting responses than lead-injected chicks (Table 1).

Recognition

In the caretaker-stranger choice test of individual recognition,

*If scores for all three treatments were similar, days were not included. Thus, if there were differences on only 13 days, only these were included in the contingency table.

tOut of 45 test days. If scores were equal it was not attributed to any treatment.

:~Out of a possible 15 trials. If scores were equal it was not attributed to any treatment.

See text for explanation of what is the best behavior pattern to exhibit.

discrimination increased over time, with almost 80% recognition by day 5, and 100% recognition by day 17 (Fig. 2). Incorrect responses involved moving to the side as often as moving to the inappropriate technician. In general, control chicks showed a significant preference for the correct stimulus (their caretaker) by day 5, whereas the 0.1 Pb mg/g birds did so by day 10, and the 0.2 Pb mg/g birds did so by day 14, $\chi^2(1) = 4.50$, $p < 0.05$.

As well as ultimately eating from the correct stimulus (their caretaker), chicks could move about before going to the stimulus. During 5 of the first 8 tests, the lead-injected chicks moved significantly greater distances than the control chicks (Table 1). That is, the control chicks walked directly to their caretaker while the lead-injected chicks wandered about.

When the cup was first removed, chicks usually looked at both stimuli (the caretaker and the other person). The number of turns made before the chick selected a direction decreased over time for all chicks (Table 2), and there was a significant treatment difference only on day 5. The number of calls given in the test situation differed significantly one day after injection, but not thereafter (Table 2).

Thermoregulation

I examined thermoregulation by providing heat-stressed chicks with a choice of going to an object that provides no shade or to a shaded area (with no object). There were no significant differences as a function of treatment when each trial was examined separately (Table 1). However, when examined over the 15 trials, the control chicks reached the shade significantly sooner, stayed there longer, and gave more contact calls (Table 1).

Depth Perception

Depth reception was measured using a modified visual cliff

apparatus (Fig. 1). There were no treatment differences in the behavior of chicks on the visual cliff apparatus on any given day (Table 1). However, when all days are examined together, the control chicks had higher scores and gave more peerings on more days than did the lead-injected chicks (Table 1).

As the herring gull chicks grew in size (to reach 700-900 g), the drop from the edge of the visual cliff became less severe and larger chicks willingly jumped off the edge. Thus, at 45 days of age, I tested them on a novel, 2 m high cliff by placing them 50 cm from the edge and presenting a fish just off the edge (to promote their approach). Performance scores were related to lead dosage, with control birds having higher scores and approaching the edge more closely than the lead-injected birds (Table 3). Only control birds succeeded in obtaining the fish, and one 0.2 Pb mg/g chick backed up so far it fell off the back of the cliff apparatus.

Incline Test and Feeding Ability

At 45 days of age, I also examined balance and locomotion by placing chicks on an incline. Although there were no treatment differences in the angle chicks fell off, control chicks walked significantly farther up the incline than did lead-injected birds.

I examined feeding ability by measuring the time to swallow two fish in succession. For both the first and second fish the time required to swallow a fish was less for control compared to lead-injected young herring gulls (Table 3).

DISCUSSION

The results of these experiments show that a single exposure of lead early in development results in defects in a variety of behavioral performances. The three doses provided clear doseresponse relationships for most test variables. Behaviors affected by lead included balance, locomotion, fighting response, ther-

Given are means \pm standard deviation.

moregulation, individual recognition, depth perception, begging behavior and fish swallowing speed. Thus, the behaviors tested ranged from simple balancing ability to complicated tasks where the bird had to find and remain in the shade.

For some of the behavioral tests there were clear, sigificant differences among treatment groups on particular days (balance score, individual recognition, incline test, visual cliff, fish swallowing). For other behaviors (begging, distance walked on balance test, visual cliff, thermoregulation) there were no significant differences on particular days. However, using the small sample sizes required of an in-depth behavioral study where animals must be tested often, it is not possible to obtain significant differences each day. Yet, if overall control birds have better scores on more days than expected by chance, then I suggest that this demonstrates subtle behavioral differences that may be critical for survival in the case of wild birds. In this study, all of these behaviors showed significant treatment effects when all tests were considered together. That is, average behavioral scores for control

accompanies large daily variances in individual behavior. Begging is a complex behavior pattern that changes with age, both in intensity and form (48). Initially, begging intensity is low, with relatively quiet vocalizations. With increasing age, begging intensity increases until chicks jump up and down flapping their wings and vocalizing loudly and continuously. Overall, the lead-injected birds continued to beg in a manner similar to the control birds, and their behavior was not distinguishable by inspection. However, begging behavior was more intense for the control birds on significantly more days than the lead-injected birds. Thus, the behavioral differences on a daily basis were subtle, but present. Even a slight difference in begging intensity might lead to problems for chicks, because they may elicit less foraging on the part of their parents, or might obtain less food in direct competition with less-impaired siblings. Sibling competition in birds can be strong and lead to differential survival (45).

Feeding behavior (fish swallowing) was only examined at 43 days postinjection and the speed of swallowing related to dose, with control birds swallowing the fastest. Swallowing speed is directly related to survival in the wild because a bird holding onto a large fish may lose it to other siblings or to pirates waiting to steal food (34). It is common in breeding colonies to see two siblings fighting over a fish or other food item.

Balance was the behavior that was most clearly affected in the five days following lead-injection. Controls had better balance than lead-injected birds, and the effects on the lead-injected birds related to dose. After eight days, there were fewer days with clear balance differences, and on 32 of 43 days the average balance scores were equivalent. This suggests that balance is initially interrupted, but the effects disappear with time, or the birds adapt. Balance is critical in nature since the chicks normally walk about when 2 or 3 days old (28), and can stumble over rough terrain, rocks or logs. Further, some gulls nest on island ledges, trees or cliffs (17,57), and must maintain their balance and not fall off the high nest sites. The righting response measured another aspect of balance, and it showed clear treatment effects with control birds

Given are means \pm one standard deviation, with Kruskal-Wallis χ^2 tests.

having lower response times on more days than lead-injected birds. Since gulls in nature frequently tumble off nests, bushes, rocks, logs and down inclines, the ability to rapidly right themselves is critical to avoiding predation by reducing their vulnerability.

I measured individual recognition by examining their responses to their caretaker. In the wild, gulls develop recognition for their parents, and this recognition occurs when mobility develops (22,28). The delayed recognition abilities of lead-injected birds would present a problem for them in the wild because recognition is essential for finding their own parents and staying on their territory. Approaching an adult that is not a parent can result in severe injury and death from the stranger (10, 27, 36), making selection strong for recognition. Although some of the leadinjected birds showed recognition early, others were delayed until 17 days. The ability to recognize parents (or in this case the caretaker) is obviously a more complex task than balance or begging behavior. The chick must pick out specific features that allow recognition and distinguishes that individual from others. These results suggest recognition of objects and people might be a worthwhile paradigm to test in human children exposed to lead. Thatcher *et al.* (62) found a negative correlation between lead and IQ and school achievement in children (but no motor deficits). Monkeys showed no deficit in learning a spatial and nonspatial matching test, but were impaired in a delayed-matching paradigm (50,55). Rats, however, showed no impairment in learning reversal tasks (33), suggesting that learning may be variable. However, in the case of the rats and monkeys the tasks they were asked to perform do not occur in the wild, and thus have not been exposed to selection. The recognition paradigm tested in this paper is directly related to the necessary parental recognition in the wild, and has undergone intense selection. The clear differences observed suggest that the features used in recognition could be profitably studied in this, and in other species.

Depth perception showed cumulative effects over time with control birds finding shade quicker, and remaining in the shade longer than lead-injected birds. Since some gulls do nest on cliffs and on trees (see above), the ability to perceive a cliff and avoid it is obviously critical to survival. Falling off a cliff or tree usually results in immediate death or abandonment by parents that concentrate on the chicks remaining in the nest.

Thermoregulation abilities are also critical to survival since some gulls frequently nest in hot environments including dry lakes and deserts (32,47) where thermal stress may be an important cause of mortality. In these tests there were no daily differences, but cumulatively the control chicks performed better on more days than the lead-injected birds. Further, the controls gave significantly more vocalizations than the lead-injected birds. Giving calls

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is adaptive because in nature it would bring a parent who would shade the chick, providing a cooler environment and leading to increased chances of survival (32).

Overall, these results indicate that there are subtle differences in behavior of controls and lead-injected herring gulls, with the controls performing the tasks better than the lead-injected birds. These tasks all relate to behavior necessary for survival in the wild and continued well-being. The results were similar, although some of the tests differed, to results obtained for common terns, *Sterna hirundo* (16). In their experiment, daily, but not cumulative effects, were examined. This difference in methodology might account for the lack of statistical differences as a function of lead dosage for begging and the incline experiment in common terns. The occurrence of similar results overall in a gull and a tern suggest that the findings may be general and indicate paradigms useful for future research.

The birds lived for 45 days, and none of them died before they were sacrificed. They did not show signs of being sick in that laboratory technicians could not distinguish the treatment groups by observation. Thus, the behavioral effects were not due to the birds being ill, certainly they were not ill for 45 days. Although the lead-injected birds were lighter, the behavioral differences were not due to weight differences since the lightest control birds performed better than the heaviest experimental birds.

Herring Gulls in nature can have lead levels of up to 20,000 ppb in liver (19). In this study lead levels in the liver at 45 for the 0.1 μ /g experimental group ranged up to 2,600 ppb (Burger and Gochfeld, unpublished data) indicating that group had levels found in nature. Since most behavioral differences were apparent in 0.1 μ g/g experimental group, I feel the differences found could correspond to those in nature.

The occurrence of conflicting results with respect to the effects of lead in humans and other animals (8,23) may be due to unstable biological criteria, inadequate biological measures, inappropriate or unnatural test paradigms, or different statistical methods. The results of this study suggest subtle behavioral differences at low-lead levels may require cumulative statistical comparisons, especially where sample sizes are small, as well as paradigms that relate to naturally occurring behavior.

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