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Behavior Effects of Lead Exposure on Different Days for Gull (*Larus argentatus*) Chicks

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BURGER, J. AND M. GOCHFELD. *Behavior effects of lead exposure on different days for gull* (Larus argentatus) *chicks*. PHARMACOL BIOCHEM BEHAV **50**(1) 97-105, 1995.—Lead exposure early in life affects behavioral, physiologic, and intellectual development in humans and other animals. In this article, we examine the effects of temporal differences in lead exposure on early development in herring gulls (*Larus argentatus*). Each of 72 1-day-old herring gull chicks was randomly assigned to one of six treatment groups to receive a lead nitrate concentration of 100 μg/g at age 2 or at age 6, a similar cumulative dose evenly divided on days 2, 4, and 6, or matched-volume saline injections on the same days. Behavioral tests were performed (some at 2- and others at 5-day intervals) to examine locomotion, balance, righting response, thermoregulation, and visual cliff. Most variation in weight was explained by testing age, although treatment affected weight gain for the lead-6 gulls, particularly after 20 days. Although treatment influenced balance and locomotion, the effect was small. The lead-6 birds were unable to remain on an incline as long as the lead-2, lead-246, and control birds. The overall score for balance improved with age for controls, showed little change for the lead-2 and lead-2-4-6 gulls, but showed a decrease in performance for the lead-6 birds. On the thermoregulation test, the lead-6 birds performed less well under both low- and high-temperature test conditions. Although the lead-2-4-6 birds had a lower score on the visual cliff tests than the other groups, the lead-6 gulls showed a significant delay in response and gave significantly fewer calls then the other groups. Overall, the data showed that the lead-6 group was more affected by the dose than the other groups, suggesting that 6 days of age may be a more critical period than earlier ages for some behaviors.

Lead Postnatal Behavioral toxicology Temporal Gulls Critical periods Behavioral development

EPIDEMIOLOGIC and experimental studies of lead toxicity in humans in the United States, Europe, and Australia have suggested that lead at low doses poses a serious threat to infants and children. The exposure to lead in childhood is associated with immediate retarded psychomotor development (1,7,15,23) and with deficits in central nervous system functioning that persist into young adulthood (5,26). There is evidence for dose-related, nonthreshold effects for children for verbal intelligence quotient, mental development, and physical development (24). Similar developmental deficits have been observed in monkeys, rodents, and birds (4,12,13,16,28).

Despite decreases in human blood lead levels in the United States in recent years as a result of decreases in the use of leaded gasoline (3), some cohorts of children are experiencing increased lead levels (2). Continued concern has resulted in the U.S. Environmental Protection Agency proposing zero levels for lead in drinking water, as well as regulating lead as a carcinogen (24). Animals that share commonalities with humans can serve as useful models to examine the effects of low-level lead exposure. In this study, we examined the effect of temporal differences in lead exposure on young herring gulls (*Larus argentatus*) to test for critical periods in neurobehavioral development.

Birds are useful models for studies of central nervous system toxicity because they rely on visual and vocal communication, which birds share with humans, unlike laboratory ro-

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dents, which rely largely on olfactory and ultrasonic modes of communication. Birds have a neonatal development period when they are dependent on their parents for protection from predators and provisioning of food. Herring gulls are ideal for these experiments because they are large, easy to raise in the laboratory, and readily adaptable to human handling, and they eat a variety of easily obtained food. Moreover, there is a voluminous literature on their behavior in the field and in the laboratory (6,8,10,22,27,32). This allows for the examination of the effects of lead on behaviors that directly relate to survival and fitness.

Lead affects schedule-controlled behavior, performance, and anatomic variables in pigeons (*Columbia livia*) (22). In previous experiments we have shown that low-level exposure to lead affects a variety of behaviors (11–13). However, in these experiments we examined the effect of two different doses administered on day 1. The present experiments followed directly, but examined the effect of timing of exposure on behavioral development. We compared the effect of a low dose administered at day 2, days 2, 4, and 6, and day 6, vs. saline-treated controls.

In previous experiments, we showed that developing common terns (*Sterna hirundo*) and herring gulls exposed to variable doses of lead administered on day 1 were adversely affected (11–13,18). Of particular interest was a lead-induced delay in parental recognition, depth perception, and thermoregulation as a function of differences in dose. All of these behaviors are essential for survival in the wild. The present experiment followed from these experiments, and examined the effect of timing of exposure, rather than dose.

METHOD

Under appropriate federal and state permits, 72 1-day-old herring gull chicks were collected from Captree, Long Island, and salt marshes in Barnegat Bay, New Jersey, in 1992. There were no differences among the chicks collected at each site, and the data were combined for analysis. Only the first hatched chick in any nest was collected to minimize effects on reproductive success. Chicks were marked with numbered leg bands for identification and randomly assigned to a treatment or control group.

Chicks were housed in groups of two or three in cages and maintained in a warm laboratory at 27 ± 2°C with a natural light–dark cycle. They were maintained in groups because in nature, gull chicks are normally in broods of two to three. Three to four times daily, they were fed a diet of high-protein cat and dog food (augmented by fish), by only one research assistant to allow for normal imprinting. This caretaker fed each chick individually until it no longer continued to eat, to ensure that no chick was deprived of food by competition with other cagemates. Chicks were fed three to four times a day.

Exposure

Chicks were either given intraperitoneal injections of lead nitrate (100 μg/g of lead in sterile water) or a normal saline solution. Injections for both control and experimental birds were given on either day 2, day 6, or days 2, 4, and 6 (hereafter referred to as 2, 6, or 2-4-6). The lead-2-4-6 chicks received the same total dose split in thirds across the 3 days, hereafter termed *exposure ages.* Control chicks were injected in the same manner as experimental chicks.

Lead injection was performed by a technician not otherwise involved in the behavioral observations, and the exposure regimen was not revealed to persons performing the behavioral tests. Exposure was by injection rather than feeding because chicks that eat different amounts would receive different doses, and a standardize dose was preferred.

Over the course of the study, some chicks died in all exposure groups. The mortality was considerably less than developing gulls experience in the wild [30–60%, depending on the year (10)]. Initially, there were 12 chicks in each of the three control groups and 12 in each of the three lead-treated groups.

Testing

Some tests were performed every other day until 28 days, and on days 34 and 42 (righting response, balance, incline). Others were performed every 5 days (visual cliff, thermoregulation). The design was balanced with all groups tested at the same ages. This combination of tests was used to evaluate balance, locomotion, depth perception, and thermoregulation. The tests that might involve habituation were performed less often. Normally, chicks were fed before tests were performed.

Although several assistants performed the tests, they were all blind with respect to the chicks' treatment. Furthermore, the same assistants performed the same tests—that is, the same two people performed the thermoregulation and visual cliff experiments, and the same two people performed the other tests to avoid interobserver variability.

Before 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. Chicks were weighed and fed. 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 10 (fell off immediately) to 1 (remained upright without using any body movements for balance). The scale included: 8 = fell off within 3 s; 6 = fell off within 10 s, waved about wildly before falling; 4 = did not fall off, but waved wings wildly to maintain balance; 2 = remained upright, used slight body movements to maintain balance.

We also tested balance by placing chicks on a board elevated at a 25° angle from the horizontal. The board was covered with sandpaper to provide traction. We recorded the distance they could move on the incline in 5 and 10 s, and the time before they fell off.

Thermoregulation was examined by placing a chick in the center of an apparatus that offered choices between full sun, a raised object that provided no shade, or a shaded area without a raised object (Fig. 1). The chicks were maintained in visual and vocal isolation until they were tested. The test ran for 2 min, and the substrate temperature was 27–29°C in the shade and 28–44°C in full sun. Data were divided into low temperatures (38–39° in full sun) and high temperatures (42–40°C) for analysis. We recorded the time for the chick to reach cover (a solid object that provided no shade), time to reach the shade (provided no cover), the total time the chick remained in the shade out of 2 min, and the total number of calls given by the test chick during the entire 2-min test. 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. Chicks were placed in the center, facing to the side where they could see both the opaque and the transparent surfaces. They remained on the test apparatus for 3 min.

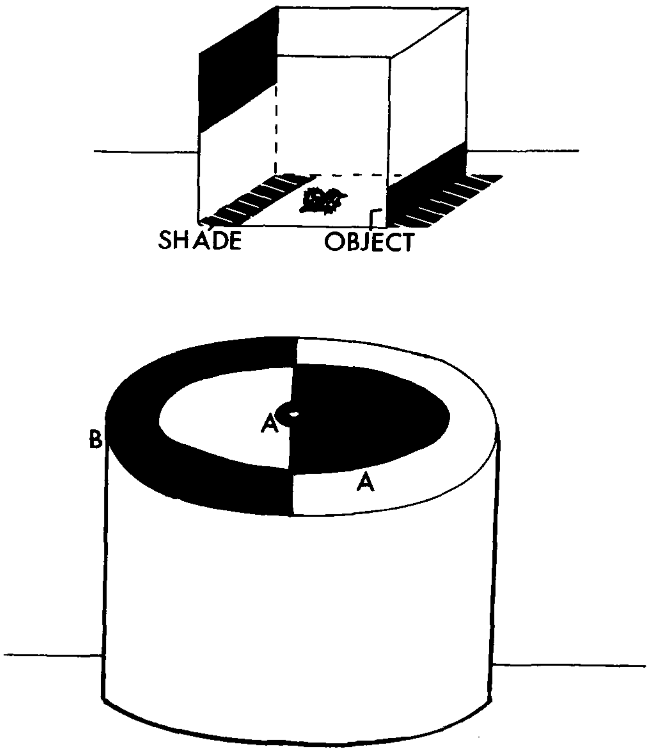

FIG. 1. Diagram of thermoregulation and visual cliff test devices.

We recorded a score for their performance and the total number of visual peerings given at the cliff edge. Peering is when the chick stops abruptly at the cliff edge with its feet at the edge and its body well back from the edge, and extends its head to peer over the edge. The chick's behavior was scored as: −1 = chick moved onto interior clear surface (fell over interior cliff); −2 = chick fell off outer cliff; −3 = chick moved onto first cliff edge and then moved onto second clear surface; 0 = chick did not move; +1 = chick moved onto and remained on interior opaque (safe) surface; +2 = chick moved onto outer opaque surface; and +3 = chick went to actual cliff edge walking only on opaque surfaces and performed one to 15 peerings at the edge.

Because the chicks were acclimated to people from day 1, they showed no signs of fear or escape behavior during any of the tests. Visual cliff, thermoregulation, and incline were performed after feeding so that the chicks were satiated and did not run to the technician. During all tests, chicks were in visual and vocal isolation from the other chicks. Whenever possible, the technicians were also hidden from view.

Statistical Tests

We used multiple regression procedures to determine whether treatment and age contributed to differences in behavioral responses (PROC GLM; 29). This provides an F statistic for the significance of the regression model, and an R^2 (the square of the multiple correlation of the reported R^2). For each contributing variable the model provides an F statistic for the contribution of that variable to the overall R^2, and the significance (p). This models procedure determines the R^2 that is contributed by each variable, adding new variables only when they increase the R^2, continuing until all variables are added. Thus, variables that vary colinearly are not added (29). We used Kruskal-Wallis χ^2 tests to examine differences among groups, followed by Duncan's multiple range test (31).

RESULTS

Weight

Seventy-seven percent of the variability in weight was explained by a model in terms of age, day of injection (exposure age), and exposure age × status (lead or control; see Table 2). Controls showed a steady increase in weight throughout the study, as did the lead-2 and lead-2-4-6 birds. However, the lead-6 group stopped gaining weight after day 20 (Fig. 2). The lead-6 birds were sacrificed on day 40 because they continued to lose weight and were in danger of dying before tissues could be sampled for lead analysis.

Righting

The balance beam and incline were used to examine balance. Lead-injected gulls (lead-2) took longer to right themselves immediately after treatment than did controls (Table 1). However, the lead-2-4-6 and lead-6 chicks did not take longer to right themselves.

We then constructed three sets of linear models for the righting and balance tests (Table 2): models for the whole data set (all groups, all ages), for days 3–12 (the period immediately following injection, when lead should have the greatest effect), and for the lead birds only (when exposure age may have a greater effect). Status refers to lead vs. control; exposure ages were 0 for all controls and 2, 6, or 2-4-6 for lead.

Variations in the righting response were explained by testing age for all the data (r^2 = 0.14), but by testing age and status × exposure age for the 3–12-day-olds (r^2 = 0.06). This indicates that testing age has an overriding effect, but when a 9-day window after injection is examined, treatment (status × exposure age) also enters as a significant variable.

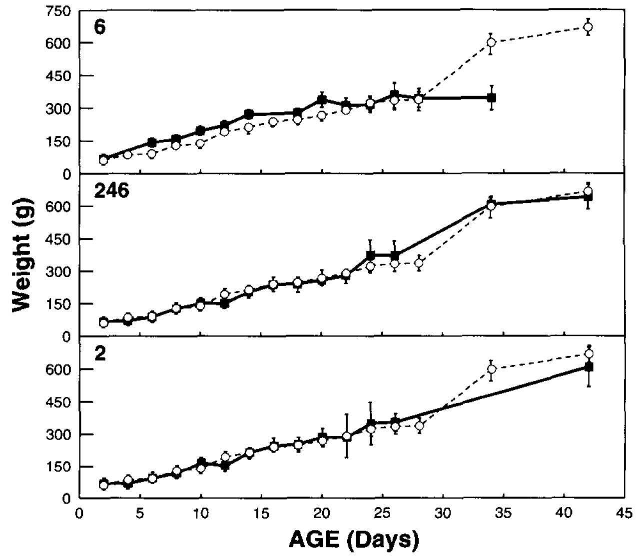

FIG. 2. Weight of control (dotted line) and lead-treated birds (solid line) as a function of age.

TABLE 1

MEAN (±SE) SCORES FOR CONTROL VS. LEAD BIRDS ON TESTING DAYS 3-12 FOR RIGHTING AND BALANCE

	Control	Lead injection days			Kruskal-Wallis $\chi^2(p<)$
		2	2,4,6	6	
Number of fat chicks	27	8	10	9	
Righting Response	1.7 ± 0.3	2.7 ± 0.8	1.6 ± 0.3	1.0 ± 0.1	13.8 (0.003)
Duncan test	A	A	A	B	
Balance beam					
Remain on beam (s)	9.6 ± 0.4	10.1 ± 0.8	10.9 ± 0.8	7.5 ± 0.8	8.9 (0.03)
Duncan test	A	A	A	B	
Distance moved in 5 s (cm)	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.3 ± 0.1	9.1 (0.02)
Duncan test	A	B	B	C	
Distance moved in 10 s (cm)	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.2	2.2 (NS)
Final score	4.1 ± 0.5	4.9 ± 0.7	6.9 ± 1.2	3.6 ± 0.4	8.7 (0.03)
Duncan test	A	A	B	A	
Incline					
Distance moved in 5 s (cm)	0.4 ± 0.1	1.2 ± 0.3	0.9 ± 0.3	0.6 ± 0.1	10.2 (0.01)
Duncan test	A	B	B	A	
Distance moved in 10 s (cm)	0.8 ± 0.2	2.0 ± 0.5	2.2 ± 0.6	1.0 ± 0.3	19.6 (0.0002)
Duncan test	A	B	B	A	
Seconds until fell off	33 ± 2	31 ± 3	34 ± 3	15 ± 2	26.5 (0.0001)

Data are given as Kruskal-Wallis χ^2 (probabilities). Shown below means are letters indicating significant differences using Duncan multiple range test.

Balance

During the balance beam test, we recorded the time they remained on the beam, the distance moved in 5 and 10 s, and a final score. Overall, there were significant differences in three of these measures (Table 1). The lead-6 birds remained on the beam for significantly less time than the others, indicating that they were more affected. The control birds seemed not to move, but remained in place, whereas the lead-treated birds moved slightly. The lead-6 birds also had significantly lower final scores than the other birds, and the lead-2-4-6 had higher final scores overall (Table 1).

Their final score reflected a steady improvement with age for the controls (scores decreased over time). The pattern for the lead birds was complex: The lead-2 birds showed a worsening of response on days 12–14, and again on days 20–22, whereas the lead-2-4-6 showed a worsening of response on days 8–18 (Fig. 3). The lead-6 birds showed no negative response until days 16 and 24–26, but the variability was quite high. Variations (r^2 = 0.02–0.08) in behaviors on the balance beam were also primarily associated with testing age, although status and exposure age entered some of the models for the data overall (Table 2).

On the incline test, the gulls moved slightly more than on the balance beam (Table 1). In most cases the gulls merely stood on the incline and looked around; because of the angle they eventually lost their balance and fell off. The pattern in time to fall indicates that the lead-2 birds suffered throughout development, the lead-6 birds showed a temporary response from days 6–16, and the lead-2-4-6 birds showed no significant response (Fig. 4).

For the 3–12-day period after injection, the controls moved the least on the incline and the lead birds moved more (Table 1). It appeared that the movement was not voluntary, but involved movements slightly up or down to maintain balance. By moving to regain balance, the lead-2 and lead-2-4-6 birds were able to remain on the incline as long as the controls; however, the lead-6 birds fell off sooner (Table 1).

Overall variations were explained by status (lead vs. control) and exposure age (for final score). The most variation in models for the different measures of balance was explained for seconds to fall off the incline. Status and exposure age entered the overall models as significant variables.

On the whole, these tests indicate small but significant differences in behavior as a function of lead treatment. The lead-6 birds showed the greatest behavioral effects and ultimately experienced a decreased rate of weight gain. The decreases in weight gain, however, occurred after day 20, whereas the behavioral deficits occurred earlier.

Thermoregulation

We examined thermoregulation by providing nonheat-stressed (sun temperatures of 38–39°C) and heat-stressed (42–40°C) chicks with a choice of going to a vertical object that provides no shade or to a shaded area (with no object for cover; Fig. 1). We then used a multiple regression procedure to examine the effect of the independent variables (Table 3). Testing age did not enter any model as a significant variable, nor did temperature or status × testing age. However, status (for time in shade), exposure age (for calls and time to cover), temperature × status (for time to cover), and temperature × exposure age (for time to cover) entered as significant variables. The most variation (R^2 = 0.21) was explained for time to reach cover, the solid object that provided visual protection but no shade.

Because ambient temperature should have affected the chicks' behavior, we examined the data by high and low temperatures (Table 4). At low temperatures only the number of calls varied significantly, and at high temperatures time to reach cover varied. However, with small samples (eight to 10 chicks per group), and the inherent variability in behavior,

TABLE 2

MULTIPLE REGRESSION MODELS EXAMINING VARIATIONS FOR ALL BIRDS, FOR ALL BIRDS (TESTING AGE 3-12 DAYS), AND FOR EXPERIMENTAL LEAD BIRDS (TESTING AGE 3-12 DAYS)

		Balance				Incline			
	Seconds to Righting	Remain On	Distance Moved in 5 s	Distance Moved in 10 s	Final Score	Distance Moved in 5 s	Distance Moved in 10 s	Seconds Until Fell Off	Weight
All data (*df* = 5,571)									
Model									
F	14.64	1.3	1.5	1.0	5.5	1.67	2.8	4.6	312
R^2	0.14	0.03	0.02	0.02	0.06	0.01	0.03	0.08	0.77
p	0.0001	NS	NS	NS	0.0001	NS	0.01	0.0002	0.0001
Factors entering									
Status $F(p)$	NS	NS	4.3 (0.03)	NS	3.5 (0.06)	5.7 (0.01)	7.5 (0.006)	17.1 (0.0001)	75.6 (0.001)
Exposure age	NS	NS	NS	NS	4.6 (0.01)	NS	NS	2.8 (0.06)	6.8 (0.001)
Status × exposure age	NS	NS	NS	NS	NS	NS	2.9 (0.06)	NS	1709 (0.0001)
Testing age	81.5 (0.0001)	2.8 (0.09)	4.3 (0.03)	4.8 (0.03)	18.8 (0.0001)	NS	NS	NS	NS
Testing age 3-12 days (*df* = 5,271)									
Model									
F	2.9	2.9	1.3	0.92	3.0	3.4	3.4	7.3	109
R^2	0.06	0.06	0.03	0.02	0.07	0.08	0.08	0.14	.72
p	0.01	0.01	NS	NS	0.007	0.003	0.002	0.0001	0.0001
Factors entering									
Status $F(p)$	NS	NS	5.3 (0.02)			10.6 (0.001)	14.5 (0.0002)	10.9	12.4 (0.0005)
Exposure age	NS	3.3 (0.01)	NS	NS	3.5 (0.009)	NS	NS	6.3 (0.001)	64.5 (0.0001)
Status × exposure age	2.4 (0.05)	NS	NS	NS	NS	NS	NS	NS	NS
Testing age	6.7 (0.009)	4.0 (0.04)	NS	NS	3.5 (0.05)	6.8 (0.009)	NS	7.4 (0.007)	383 (0.0001)
Experimental (Lead, *df* = 2,145)									
Model									
F	2.0	3.6	0.7	1.4	3.5	2.5	1.2	9.2	128
R^2	0.04	0.08	0.02	0.03	0.08	0.05	0.03	0.16	0.73
p	NS	0.02	NS	NS	0.01	0.06	NS	0.0001	0.0001
Factors entering									
Exposure age $F(p)$	2.4 (0.09)	4.2 (0.01)	NS	NS	5.0 (0.008)	NS	NS	11.1 (0.001)	106 (0.0001)
Testing age	NS	NS	NS	4.11 (0.04)	NS	5.8 (0.01)	NS	5.3 (0.02)	170 (0.0001)

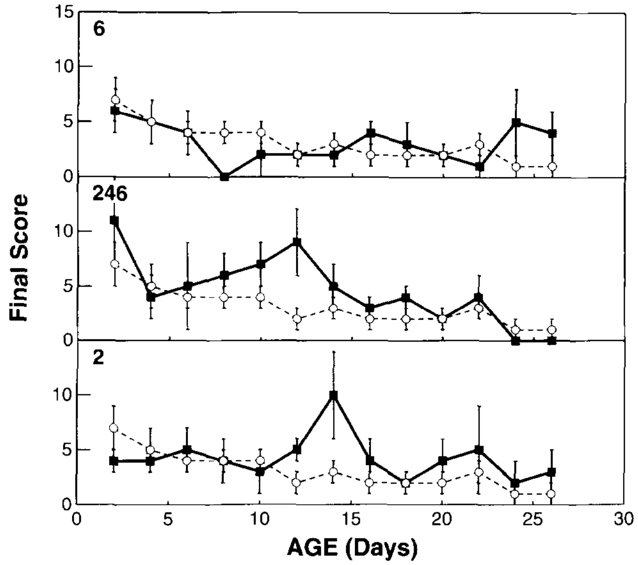

FIG. 3. Final score on balance beam test for control (dotted line) and lead-treated gulls (solid line) as a function of age.

lack of differences in distributions is not surprising. However, in six of the eight comparisons, the lead-6 birds performed less well than all other groups (contingency $\chi^2 = 5.3$; $p < 0.05$). Similarly, in comparing the lead-6 gulls with each of the three groups for the eight comparisons of Table 4, the lead-6 gulls performed less well on 21 of 24 possibilities (contingency $\chi^2 = 61.6$; $p < 0.001$). Thus, overall, the lead-6 birds performed much less well than all other groups with respect to thermoregulation.

Visual Cliff

Models for behavior on the visual cliff generally explained only 1–8% ($p < 0.05$) of the variation in terms of exposure age for all except the number of peerings. The total score, time to respond, and number of calls all differed among groups (Table 5). On the whole, the total score for the lead-2-4-6 group was significantly lower than for the others. A higher proportion of the lead-2-4-6 birds moved onto the clear surface where they would have fallen off the cliff (Fig. 5). The controls and the lead-6 gulls remained near where they were placed, whereas the other lead birds moved more, either to go off the cliff (lead-2-4-6) or onto a safe area (lead-2). Thus, it appears that lead at age 2 days results in stimulating activity, whereas the lead-6 and control birds did not move.

The lead-6 birds took significantly longer to respond (move in any direction), and called less than the gulls in the other groups (Table 5). Thus, the lead-6 group showed a delayed response in both moving and calling.

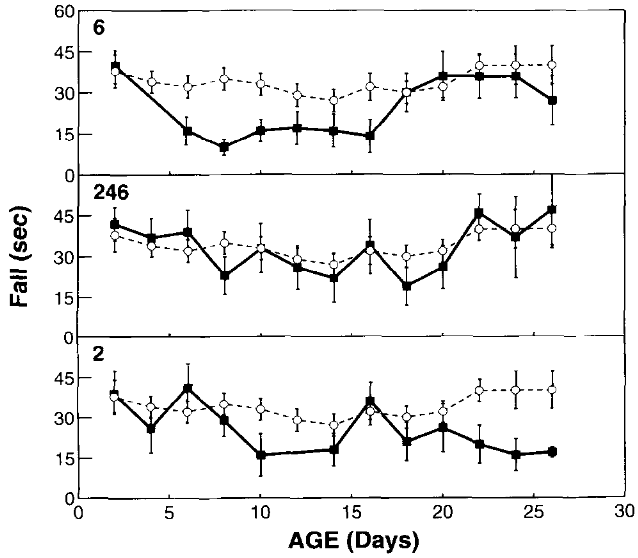

FIG. 4. Time gulls fell off incline for control (dotted line) and lead-treated groups (solid line) as a function of age.

DISCUSSION

Methodologic Considerations

One potential problem is the possibility of lead exposure from the female via the egg, because the young were collected from the wild. However, there is no reason to assume that such exposure would vary consistently among groups. Second, the chick grows so much during development that any exposure from the egg is diluted markedly in the young.

Experimentation with behavioral effects may be difficult because many behaviors are variable, some are influenced by motivation, and effects may be subtle. Moreover, many behavioral experiments are often time-consuming. However, behavioral change may be a sensitive end point for certain toxics, particularly as normal behavior may be critical for survival (see subsequent discussion).

Although some of the R^2 results were low for our models, treatment (status or exposure age) entered most models as a significant variable accounting for variations in a wide array of behaviors. This overall finding is important because it indicates that lead affects several aspects of behavior.

We also note three other problems. First, several assistants were involved in these experiments. Because the gulls became familiar with the assistants, they often moved toward them, or where they last saw them. In some tests this may have confounded the direction of their movement. In nature, gulls regularly seek their parents or cover when left alone (10). We tried to eliminate the effect of several assistants by having the same assistants perform the same tests each day, and by having these assistants blinded as to treatment groups.

Second, on the visual cliff, chicks could test the surface by gently moving their foot onto the clear plastic (the cliff) and ascertaining that they would not fall off. Thus, some tests scored as negative may in fact have been positive. Nonetheless, we used a criterion of where they stepped because it was easily distinguished and was repeatable. Even with these difficulties there were significant, and generally consistent, results across tests.

Third, although we gave the lead-treated birds equivalent dose injections, individual variations can result in differences in brain lead levels. These differences in brain lead levels may have accounted for some of the variations in behavior observed in this study. Tissues have been archived for analysis of lead levels, and preliminary data indicate that there is a significant correlation between brain lead levels and some of the behavioral measures (unpublished data). These data will be published elsewhere after complete analysis of a range of tissues.

TABLE 3

MODELS EXPLAINING VARIATIONS IN THERMOREGULATORY BEHAVIOR

	Time to Cover	Time to Shade	Time in Shade	Number of Calls
Model				
F	7.0	0.8	1.2	2.2
R^2	0.21	0.03	0.05	0.08
p	0.0001	NS	NS	0.03
Factors entering*				
Status $F(p)$	3.4 (0.06)	2.97 (0.08)	4.0 (0.04)	NS
Exposure age	6.8 (0.001)	NS	NS	6.8 (0.001)
Temperature × status	5.1 (0.007)	NS	NS	NS
Temperature × exposure age	9.9 (0.0001)	NS	NS	NS

*Variables of ambient temperature, testing age, and status × exposure age interaction had no significant independent contribution to any of the dependent variables.

Lead and Behavior

The results clearly indicate differences in behavior among groups. For many behaviors the differences were small. However, small differences in behaviors such as the ability to right themselves when they fall down sand dunes, to maintain balance on their nest or surrounding areas, and to respond quickly when left alone, influence survival in nature. Young gull chicks must learn to seek shade, seek cover, evade intruders, and maintain their balance as they move about their territory. Slight differences in their ability to respond quickly can lead to differential predation rates or susceptibility to heat stress (8,9,17,27).

Some results need an explanation in a naturalistic context. On the balance beam the lead birds generally moved about more than the control birds. However, they moved about to regain their balance, and often fell off while doing so. On the contrary, the control birds stayed where they were, did not move while trying to maintain balance, and remained on the beam.

On the incline, the control birds also moved less while remaining on the board. The lead-2 and lead-2-4-6 chicks moved while attempting to maintain balance and managed to remain on it as long as the controls. Thus, they appeared to compensate by moving. The lead-6 birds also moved, but did so less and fell off earlier.

Thermoregulation is important because some nesting habitats of gulls are extremely hot and dry (20,21,25), and others (such as sandy beaches) can be very hot at midday (14). When parents leave chicks to fly to feeding areas or to defend the territory against intruders, young chicks should immediately seek cover or shade. Furthermore, an ability to perceive available cover is critical to avoid predators that cruise over gull colonies searching for unprotected prey.

On the thermoregulation test the lead-2-4-6 birds performed more like the controls than the lead-2 or lead-6 birds.

TABLE 4

BEHAVIOR OF HERRING GULLS ON THERMOREGULATION TEST

		2 days	2,4,6 days	6 days	All groups Kruskal-Wallis χ^2 (p)
Low temperature (38-39°C)					
Time to cover (s)	0.8 ± 0.1	0.3 ± 0.2	0.7 ± 0.3	0.9 ± 0.3	2.3 (NS)
Time to shade (s)	36 ± 5	41 ± 13	33 ± 10	46 ± 10	0.4 (NS)
Time in shade (s)	60 ± 7	54 ± 10	65 ± 10	45 ± 10	2.6 (NS)
No. of calls	35 ± 5	46 ± 10	42 ± 9	10 ± 5	12.0 (0.003)
Duncan test	A	A	A	B	
High temperature (42-40°C)					
Time to cover	0.7 ± 0.1	0.8 ± 0.3	0.8 ± 0.4	3.1 ± 0.7	13.4 (0.003)
Duncan test	A	A	A	B	B
Time to shade	27 ± 4	41 ± 11	42 ± 9	34 ± 7	3.5 (NS)
Time to shade	60 ± 7	37 ± 9	50 ± 11	48 ± 10	4.7 (NS)
No. of calls	41 ± 5	38 ± 9	49 ± 10	15 ± 10	2.7 (NS)
Kruskal-Wallis χ^2 (p) and high temperature					
Time to cover	0.7 (NS)	0.1 (NS)	0.1 (NS)	10.5 (0.001)	
Time to shade	1.4 (NS)	0.1 (NS)	0.5 (NS)	0.0 (NS)	
Time in shade	0.0 (NS)	2.8 (0.09)	2.3 (NS)	0.2 (NS)	
No. of calls	0.0 (NS)	2.0 (NS)	0.3 (NS)	2.9 (0.09)	

Data shown for significant difference are the Duncan multiple range test (letters indicate lack of overlap).

TABLE 5

RESPONSE OF HERRING GULLS ON VISUAL CLIFF

	Total Score	Number of Peerings	Time to Respond (s)	Calls
Controls	0.1 ± 0.1 (C)	3.9 ± 0.3	18.6 ± 3.1 (A)	1.4 ± 0.2 (A)
Lead-2	0.4 ± 0.2 (A)	4.0 ± 0.5	14.1 ± 5.9 (A)	1.5 ± 0.2 (A)
Lead-2-4-6	−0.4 ± 0.2 (B)	4.2 ± 0.6	12.1 ± 4.2 (A)	1.5 ± 0.1 (A)
Lead-6	0.1 ± 0.2 (C)	4.0 ± 0.4	31.3 ± 5.8 (B)	0.7 ± 0.2 (B)
Kruskal-Wallis (χ^2)	15.3	0.3	15.1	15.3
p	0.0002	NS	0.002	0.001

Data given are means ± SE (letters indicate significant differences using a Duncan multiple range test).

At least on this test the cumulative dose had less effect than the single equivalent dose.

Some gulls nest on cliffs or in trees (19); thus, an ability to recognize and avoid a dropoff may lessen injury or death. On the visual cliff, the lead-2-4-6 birds had the lowest scores. However, it appears that the lead-2 and lead-2-4-6 birds responded quickly and moved, whether to the safe side (the lead-2 group) or the cliff (lead-2-4-6). The controls responded as quickly, but chose to remain relatively close to their original position. The lead-6 birds also remained near this position, but only because they took longer to respond, and so could not encounter the far cliff. Thus, knowing the mean scores alone does not provide the complete picture. The distribution of responses gives a more accurate portrayal.

Exposure Age

As early as 1962, Scott (30) hypothesized that there were critical periods in development. Previous work with terns and herring gulls indicated significant differences in behavioral responses as a function of dose. Herring gulls that received a dose of 0.02 compared with 0.01 mg/g lead at 2 days of age took significantly longer to recognize their caretaker, performed fewer begging behaviors, and had lower scores on the balance beam, but did not show differences in performance on an incline or on a visual cliff apparatus (11). Begging behavior, however, was differentially affected in that herring gulls that received the lower dose performed significantly more begging displays than control gulls, yet continued to lose weight (11,13). It seems that the more they lost weight, the more vigorously they begged to obtain more food. However, they did not eat less (13).

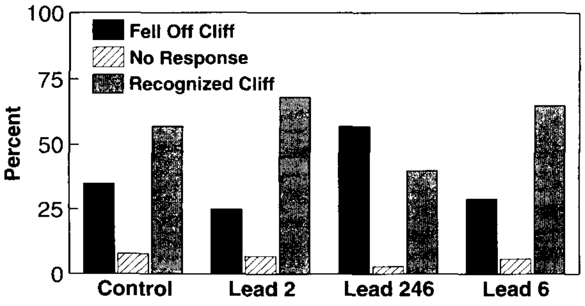

FIG. 5. Response of gulls to the visual cliff.

In this experiment we examined the effect age of exposure (2 and 6 days) and split (one dose vs. three) on behavior. There were significant differences with respect to weight and nearly all behavioral tests. Except for the righting response, the lead-6 birds performed less well on many tests. On the balance beam the lead-6 birds remained on the beam for less time, moved about trying to maintain their balance, and had lower scores than all the other groups. On the incline the lead-6 gulls fell off earlier than birds in all other groups. On the thermoregulation test, the lead-6 gulls gave fewer calls at low temperatures and required more time to reach cover than the other groups. On the visual cliff test the lead-6 birds had equivalent scores, but called fewer times and took much longer to respond than did the gulls in the other groups.

We had initially hypothesized that there might be a difference in the effect as a function of age of exposure. We had expected that lead exposure at 2 days might have a greater effect than exposure at 6 days because it is earlier in development. However, our experiments indicate that the birds injected at 6 days were significantly more impaired with respect to some behaviors.

It is also noteworthy that the lead-6 birds experienced a cessation of growth after 20 days, when the other lead groups were showing no further effect. The cessation of growth occurred 2 weeks after the behavioral affects were clear. These lead-6 birds continued to show no weight gain until 40 days, when they were sacrificed to ensure that we could acquire tissues for metal analysis. The lack of weight gain suggests they may have had absorption problems, as suggested by Cory-Slechta et al. (16).

Our results suggest that there may be a critical period for lead during behavioral development. This critical period may fall between days 2 and 6. More experimentation is required to determine whether the effects will continue to increase after day 6, or whether they will decrease. However, these differences might also be due to differences in brain lead concentrations at different ages, which also bears examination.

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