

Operant Behavior in Transition Reflects Neonatal Exposure to Cadmium

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ABSTRACT Male Long-Evans rats were injected with 0, 1, 3, or 6 mg/kg of cadmium chloride on the first day of life. Animals free of morphological stigmata at weaning were selected for study. Tissue concentrations of cadmium and operant behavior under various fixed-ratio (FR) schedules of reinforcement were evaluated when these rats were adults. Dose-related increases in cadmium were present in the brains, livers, and kidneys. Dose-related differences in behavior were most evident during the transition from fixed ratio 25 (FR 25 or 25 responses/reinforcer) to FR 75. An inverted U describes the relationship between response output during the transition to FR 75 and cadmium chloride dose: response output increased at 3 mg/kg and decreased at 6 mg/kg. The rate decreases were not correlated with weight loss that appeared after some of the animals exposed to 6 mg/kg reached 60 days of age. Challenge doses of d-amphetamine revealed no interaction between neonatal exposure to cadmium and d-amphetamine. The occurrence of alterations in operant behavior in animals that appeared normal on a number of preweaning evaluations suggests that operant behavior in transition was sensitive to subtle effects not observed with other commonly used tests. The data provide evidence for delayed effects in the adult that are due to neonatal exposure to cadmium.

Cadmium toxicity has usually been associated with the kidney and lung, and, in rodents, the testes and placenta (Klaassen, '80; Levin et al., '81; Miller and Kellogg, '85; Parizek, '64). However, accumulating evidence from several sources suggests that exposure to cadmium can also induce nervous system damage under certain conditions. This is not surprising, since other metals, e.g., mercury, lead, manganese, aluminum, and tin, produce behavioral and central nervous system (CNS) toxicity. Cadmium poisoning is anomalous because although inorganic cadmium does not enter the mature CNS, it enters the CNS of the developing animal with relative ease and may cause widespread morphological and neurochemical damage (Gabbiani et al., '67; Klaassen and Wong, '82; Webster and Valois, '81; Wong and Klaassen, '80).

The sensitivity of the developing nervous system to cadmium is reflected in behavior. Developing rats administered doses of cadmium comparable to those that cause neuropathology are impaired in learning and in visual-discrimination tasks (Winneke et al., '83). Similar doses administered to preweaning rats alter locomotor activity during both development (Ruppert et al., '85) and adulthood (Smith et al., '82). In contrast, rats exposed to cadmium only as adults tolerate much higher dose levels (Nation et al., '83).

The behavioral, morphological, and neurochemical data all indicate that cadmium can

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be neurotoxic if it penetrates the nervous system. Two possible routes of entry have been described: Inorganic cadmium penetrates an immature blood-brain barrier (Gabbiani et al., '67; Klaassen and Wong, '82; Webster and Valois, '81; Wong and Klaassen, '80) and cadmium forms a lipid-soluble complex with the chelator, diethyldithiocarbamate, which enters the mature brain (Cantilena et al., '82; Gale et al., '82). Behavioral measures play a unique role in evaluating CNS toxicity because they offer effective ways of monitoring the progressive changes that might follow exposure during development or after chelation therapy.

The present report is the second part of a study designed to evaluate the long-term consequences of a single exposure to cadmium at 1 day of age. Groups of 1-day-old rat pups were exposed to cadmium and followed throughout life. Hydrocephalus and associated disruptions on several preweaning evaluations appeared in a subpopulation of these animals (Newland et al., '83). The present report emphasizes those animals that appeared normal on all evaluations performed before weaning. A longitudinal design allowed several aspects of cadmium toxicity to be described and permitted comparisons among different tests.

Operant behavior was chosen as the endpoint for adult evaluations because it can reveal subtle effects of exposure to toxicants (Weiss, '83). Because operant behavior provides a mechanism for adapting to changes in the environment (Skinner, '81), a disturbance in such behavior represents a significant functional deficit. Behavioral adaptation to shifts in reinforcement contingencies, the essence of learning, is particularly sensitive to drugs (Thompson and Moerschbaecher, '79) and toxic substances such as lead (Cory-Slechta, '84; Mele et al., '84). The current experiment focused on the acquisition and maintenance of fixed-ratio responding. This performance was selected because a stable baseline appears quickly and the pattern of responding engendered by this schedule has been described extensively (e.g., Ferster and Skinner, '57; Weiss and Gott, '72).

METHOD

Subjects

The subjects were male Long-Evans rats maintained on a lifelong diet of tapwater and the American Institute of Nutrition (AIN) semipurified rat diet (U.S. Biochemicals). All

rats were housed in plastic cages with Pine-Dri shavings in a room with a 12-hr light-dark cycle.

The subjects were bred from Long-Evans rats obtained as weanlings from the Charles River Breeding Laboratories (Wilmington, MA). All female breeders were maintained on a diet of tapwater and the AIN semipurified diet from the day that they arrived at the laboratory until the subjects were weaned.

On postnatal day 1 (PN 1, birth = PN 0) the litters were culled to eight pups (four males and four females when possible); neonates were tattooed on their footpads for identification and injected subcutaneously with 1 ml/kg of saline or CdCl₂ dissolved in saline. The solutions were contained in coded vials in order to keep the technician performing the injections and early tests blind to treatment. When two dams delivered within 12 hr of one another, four pups were removed from their maternal dam and fostered with the other dam. Each foster litter contained pups from each of four dose groups. Thus, in the first experiment, each litter contained two pups from each dose group (0, 1, 3, and 10 mg/kg CdCl₂, calculated as the salt). All pups exposed to 10 mg/kg CdCl₂ died before weaning. In the second stage, the pups were treated as in the first stage except that each foster litter contained two pups exposed to the vehicle and six pups exposed to 6 mg/kg CdCl₂.

The rats in the first group were males receiving 1 mg/kg CdCl₂ (8 rats from 6 dams), and 3 mg/kg (6 rats from 5 dams) co-reared with rats injected with saline alone (6 rats from 4 dams). This group began operant training at 60 to 62 days of age.

The second group contained rats exposed to 6 mg/kg CdCl₂ (6 rats from 4 dams) co-reared with rats receiving saline alone (8 rats from 6 dams). The second group began operant training between 70 and 90 days of age, after the first one finished. The high-dose group contained eight rats originally, but one died shortly after operant training began and a second stopped responding shortly after the transitions began. Data from these two rats were included in the analysis of weight gain but not in the behavioral evaluations.

Before weaning, all rats were evaluated on tests of suckling and preference for home bedding (Newland et al., '83). The rats exposed to 6 mg/kg CdCl₂ were selected from a group of 24 rats, of which 11 displayed retarded weight gain and overt signs of hydro-

cephalus. The cadmium-exposed rats in the present experiment displayed no overt signs of hydrocephalus and no retardation in weight gain at weaning. That is, the rats exposed to 6 mg/kg CdCl₂ resembled their controls on all evaluations conducted before weaning (Ng et al., '84).

Apparatus

Four Lehigh Valley (Fogelsville, PA) operant chambers, each containing one lever and one fluid dipper, were used. The lever was positioned 5.5 cm above a metal grid floor. The force required to press the lever fell between 0.15 and 0.20 N. A volume of 0.1 ml of sweetened condensed milk diluted with water (water:milk = 1:1) was the reinforcer. Behavioral sessions were controlled by SUPER-SKED system on a PDP-8 computer (Snapper et al., '82). All session interevent times were collected with a resolution of 0.01 sec and stored for later analysis.

Fixed-ratio acquisition and performance

All but two rats were maintained at a body weight of 300 g, because experience in this laboratory has shown that rats at this weight respond well for sweetened condensed milk. The exceptions, both of which had received 6 mg/kg CdCl₂, developed free-feeding body weights of less than 300 g and so were maintained at lower weights (220 g and 280 g). The feeding schedule was changed for the rats exposed to 6 mg/kg CdCl₂ and their concurrent controls at the end of the experiment to assess whether the observed behavioral effects were related to body weight differences.

The rats were water-deprived early in the morning and tested in the afternoon 5 days a week. After preliminary training, all sessions were 30 min in length. Four different operant chambers were used, and animals were assigned to a chamber and session time such that the assigned doses were distributed across chambers and times.

Standard manual methods were used to train the rats to press the lever. They were then exposed to a fixed-ratio 1 (FR 1) schedule of reinforcement (one response produces access to reinforcement) for 30-min sessions. After all rats had emitted at least 100 responses during a session under the FR 1 schedule, the group was exposed to the sequence of conditions described in Table 1. During extinction (EXT) all conditions were the same except the milk reservoir was empty. One of the 6-mg/kg rats quit respond-

TABLE 1. Sequence of conditions used to evaluate schedule performance

Session	Schedule
1-5	FR 1
6-8	FR 5
9	EXT
10	FR 1
11-12	FR 25
13-30	FR 75
31-39	d-Amphetamine testing
40-42	Change feeding schedule

ing after the FR parameter was raised to 75, so the FR parameter was subsequently lowered for this animal in order to regain responding for later d-amphetamine testing.

An increasing series of d-amphetamine sulfate injections (saline, 0.3, 1.0, 3.0 mg/kg, calculated as the salt) began after responding under the largest FR schedule had stabilized, i.e., when postreinforcer pausing and response rate showed no systematic day-to-day fluctuations. The drug was dissolved in sterile saline solution such that 1 ml/kg of body weight was injected. The injection was administered IP, 10 min before the start of the session. To eliminate any novelty effect of the drug, a postsession dosage of 0.3 mg/kg of d-amphetamine was administered at least two sessions before the series of d-amphetamine treatments began.

Tissue cadmium determination and pathology

At the end of the experiment the animals were killed with carbon dioxide and samples of brain, liver, and kidney were examined for gross lesions. Kidneys, liver, and half-brain were collected for tissue analysis of cadmium content and histopathological examination. Gross and microscopic examinations were accomplished by an observer unaware of their treatment.

The tissues destined for cadmium determination were frozen at -20°C. About 100 mg of tissue was weighed and dissolved in a perchloric acid mixture (conc. HNO₃, conc. H₂SO₄) and heated overnight to dryness. The samples were then redissolved in 5 ml of 1% HNO₃ and analyzed on a Hitachi model 170-70 Zeeman atomic absorption spectrophotometer. The cadmium tissue content is expressed as nanograms cadmium per gram wet weight of tissue.

Both gross and light microscopic examination of the brains were performed. Histological examination by light microscopy was undertaken on transverse sections that in-

cluded cortex, the ventricles, and the brainstem. A subject was rated as having frank hydrocephalus if the head was dome-shaped, i.e., if hydrocephalus was evident before necropsy. It was rated as being mildly hydrocephalic if enlarged ventricles or thinned cortex was evident on gross histopathological examination, but a dome-shaped head was not noted prior to necropsy. A subject was scored as normal if there was no evidence of ventricular dilation, thin cortex, or hemorrhage.

Statistical analyses

To determine whether differences in weight gain occurred, a growth curve analysis was performed as described by Rogosa et al. ('82). A quadratic equation was fit by least squares to the successive body weights measured for each animal. Each coefficient (intercept, linear term, quadratic term) for a single animal constituted a datum so the comparison between control and exposed animals could be accomplished by comparing these terms using standard inferential statistics. One advantage of this technique is that it tracks specific features of change in individual animals. Differences in overall weight appear in the intercept, differences in the rate of growth appear in the slope, and deviations from linearity or reversals in weight gain appear in the quadratic term.

For example, the intercepts for all animals exposed to 6 mg/kg can be compared as a group with the intercepts from their concurrent control group by means of a *t* test. An ANOVA or multiple comparison techniques can be employed to reduce the probability of Type I errors incurred when performing multiple comparisons. Such adjustments were not required in the analysis of growth because all *p* values were greater than 0.20 for multiple *t* tests.

A repeated-measures analysis of variance using the program BMDP2V (Dixon and Brown, '79) was applied to evaluate dose-related differences in the acquisition and maintenance of fixed-ratio performance. The degrees of freedom were adjusted by the Greenhouse-Geisser correction before a *p* value was estimated. The logarithm of the number of responses during a 30-min session (overall response rate) was the dependent variable used for statistical testing. The data were log-transformed because variability was related to the total number of responses. Dosage was a between-subject variable and transition type was a within-subject variable. A

repeated-measures analysis of variance was also employed to evaluate the interaction between d-amphetamine and cadmium. When there was a significant overall effect (at $p < 0.05$) multiple comparisons were performed by the Newman-Keuls procedure (Kirk, '68).

A two-way analysis of variance (dose \times tissue) was applied to the logarithm of the tissue concentrations. Newman-Keuls multiple comparisons were applied when there was a significant overall effect (at $p < 0.05$).

RESULTS

Body weights

Table 2 shows mean body weights for each dose from birth until food intake was limited at PN 70. The intercept, slope, and quadratic components of the growth curves from the cadmium-exposed animals did not differ from controls (all $p > 0.20$). Thus, there were no differences in the growth of the animals. Because these results come from growing animals, they do not reveal the fact that at PN 60 three of the 6-mg/kg rats began to lose weight. When operant testing began two of these animals were lighter than the others and the third died.

Fixed-ratio performance

Training was routine for the control rats and those exposed to 1 and 3 mg/kg CdCl₂, but several of the rats exposed to 6 mg/kg CdCl₂ were difficult to train. Observations of the animals in the operant chambers suggested that the training difficulties were due to hyperresponsiveness to the noise of the dipper. All rats eventually did lever-press reliably, meeting the criterion of at least 100 responses during a 30-min session on a FR 1 schedule of reinforcement, and were exposed to the sequence of schedule values shown in Table 1. The groups did not differ in their response rates during the last session at FR 1 (Fig. 1, left).

Differences in the groups did not appear until the shift from FR 25 to FR 75 (dose \times transition interaction, $F(20,145) = 1.84$, $p = 0.04$). During this transition the group exposed to 3 mg/kg CdCl₂ responded at a higher rate than controls and the 6-mg/kg group responded at lower rates (Fig. 1). The 1-mg/kg group was intermediate between control and 3-mg/kg values but not significantly different from either.

Responding was allowed to stabilize before initiation of the d-amphetamine challenges. Final response rates for the 3- and 6-mg/kg groups differed from each other but not from

TABLE 2. Mean body weights (grams) of male rats from birth to restriction of food at PN 70¹

Dose of CdCl ₂ ²		Age (days)								
		1	5	10	15	21	30	40	50	70
Control	1	7.4 (0.2)	14.2 (0.9)	27.5 (1.4)	32.2 (6.5)	59.7 (1.4)	104.0 (1.8)	176.0 (5.0)	214.0 (14.0)	268.0 (12.9)
1 mg/kg	(N=8)	6.9 (0.2)	13.4 (0.7)	27.0 (1.0)	36.9 (1.1)	57.8 (1.5)	98.5 (2.6)	167.0 (6.5)	192.0 (14.7)	248.9 (8.4)
3 mg/kg	(N=6)	6.9 (0.3)	12.9 (0.8)	27.2 (1.2)	37.3 (1.4)	57.8 (1.8)	98.8 (3.5)	169.0 (8.5)	183.0 (17.5)	262.0 (15.1)
Control	2	6.5 (0.1)	12.1 (0.3)	23.1 (0.5)	31.8 (4.6)	49.4 (1.1)	95.3 (2.9)	181.0 (5.6)	261.0 (11.8)	319.0 (10.3)
6 mg/kg	(N=8)	6.3 (0.2)	12.4 (0.4)	22.8 (0.5)	34.4 (0.9)	47.8 (2.1)	91.1 (0.9)	161.0 (0.6)	240.0 (17.0)	323.0 (17.1)

¹The standard error of the mean is in parentheses.

²Control group 1 was co-reared with the 1- and 3-mg/kg animals. Control group 2 was co-reared with the 6-mg/kg animals.

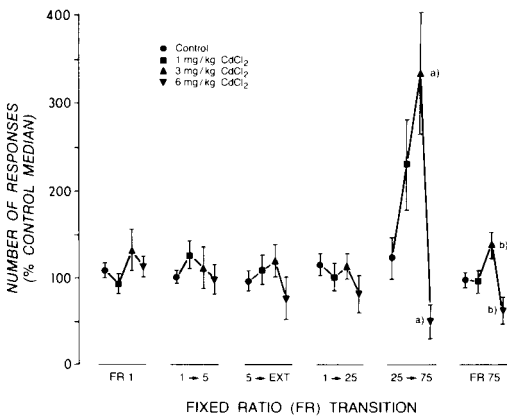


Fig. 1. Mean number of responses (± 1 SEM) during a session as a percentage of the median obtained from a concurrent control group for the last FR 1 session, four transitions, and the 19th FR 75 session. The rats exposed neonatally to 1 and 3 mg/kg CdCl₂ were compared with a concurrent control group and those exposed to 6 mg/kg CdCl₂ were compared with a second concurrent control group. The error bars for the control groups were obtained by computing the rate for each control rat as a percentage of the control median, pooling both control groups, and then obtaining a standard error of the mean in the conventional manner. Points labeled with a *a* are significantly different from control values based on Newman-Keuls comparisons of the raw response rates. In addition, the 3- and 6-mg/kg points for the last FR 75 session (labeled with a *b*) differ from each other. The median control values for the control group concurrent with the 1- and 3-mg/kg rats were 127, 391, 262, 1747, 898, and 3802 responses per session for the FR 1, the four transitions, and the FR 75 session. Those values for the control group concurrent with the 6-mg/kg rats were 162, 768, 360, 2197, 1950, and 4013 responses per session.

controls (Fig. 1, far right). The final schedule for one of the 6-mg/kg rats (second from top in column 2 of Fig. 3) was shifted to FR 15 3 days after the transition to FR 75 because it

responded at such a low rate that it collected no reinforcers under the FR 75 schedule. Shifting to an FR 15 schedule kept this rat on a fixed-ratio schedule of reinforcement adjusted such that the reinforcement rate was high enough to maintain responding. This rat's response rates remained low and in that respect it resembled other rats exposed to 6 mg/kg. The adjustment is relevant only to the FR 75 steady-state and d-amphetamine results.

The clearest effects of cadmium on the last transition appeared in the running rates, defined as the rate of responding calculated from the time between the first response after the reinforcer and completion of the fixed-ratio requirement. Figure 2 demonstrates the stabilization of the running rates for control and 3-mg/kg animals. The running rates of the 3-mg/kg animals exceeded control rates during the first FR 75 session; two animals performed within control ranges, whereas the other four ranged from four to over seven times the control median on the day of the transition. The difference in response rates declined over the succeeding sessions. Four sessions later, the running rates of both groups were higher than during the first day of transition. The running rates of the rats exposed to 3 mg/kg still exceeded controls, but more overlap is apparent. After 19 sessions the rates of the exposed animals matched control values more closely.

The cumulative records in Figure 3 demonstrate the nature of responding under the FR 75 schedule for control and high-dose rats on the fifth day of FR 75. This day was selected because it was the first day that all of the control animals demonstrated response patterns commonly seen under FR schedules: Reinforcer delivery was followed by a pause

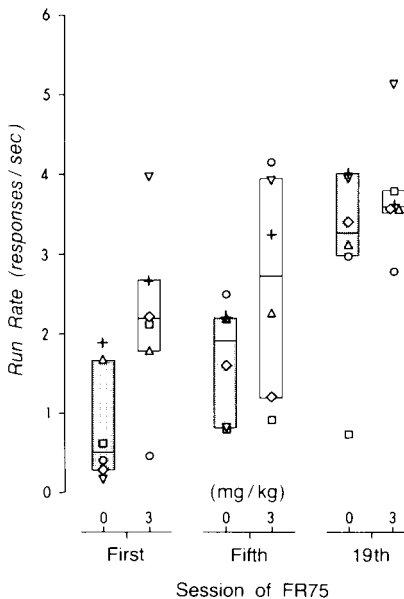


Fig. 2. Running rates for animals exposed postnatally to 0 and 3 mg/kg CdCl_2 . Rates from three stages of the last transition are shown for individual animals. Six animals were exposed to each dose and each animal within a dose is represented by a different symbol. Boxes enclose the first and third quartiles, and the median is represented by a horizontal line within the box.

after which a steady, high rate of responding occurred until the delivery of the next reinforcer. The control rats varied in both the length of the pause and the rate of responding, but the characteristic FR pattern is seen in all these rats. The four low-rate 6-mg/kg animals performed much more erratically. No clear delineation of pausing and responding was evident in these animals; instead, they demonstrated irregular sequences of pausing and responding. The top record in the 6-mg/kg column represents the rat whose performance prevented a shift to FR 75. His data were not included in the statistical analysis. No clear relationship among response rate, hydrocephalus, and body weight emerged.

d-Amphetamine effects

The effects of d-amphetamine were evaluated by expressing overall response rates as a percentage of the rate during the session on the previous day. A repeated-measures analysis of variance performed on the percent-of-control dependent measure revealed a significant effect of d-amphetamine ($F(3,90)$

$= 43.11$, $p < 0.0001$); a slight rate increase appeared at lower doses and low rates appeared at higher doses. No effect of cadmium ($F(3,30) = 1.24$, $p = 0.313$) on the percent-of-control measure and no interaction between cadmium and d-amphetamine ($F(9,90) = 1.16$, $p = 0.330$) appeared.

Evaluation of deprivation

The low body weights of three of the 6-mg/kg rats (two completed the experiment and one died in an early stage) raised a concern that the low response rates observed in this group had resulted from inadequate food deprivation. To examine the possibility that the 6-mg/kg animals were inadequately deprived, they were further deprived for 3 days. Concurrently, to determine the effect of overfeeding, the control animals were free-fed for 3 days. Water deprivation remained as before. This change in the feeding schedule resulted in an average weight loss of 15 g for the 6-mg/kg rats and an average weight gain of 65 g for the controls.

A correlated t test revealed that there was no effect of the change in the feeding regimen, either for the 6-mg/kg group ($t(5) = 1.22$, $p = 0.28$) or for the controls ($t(7) = 1.35$, $p = 0.22$).

Tissue concentrations and pathology

Postmortem examination of the brains of the adult animals revealed two cases of hydrocephalus in the 6-mg/kg group that were not evident previously. Figure 4 shows a section of one of these mildly hydrocephalic brains compared with a case of frank hydrocephalus and a control brain. Note that the brains from the 20-day-old, frankly hydrocephalic rat possessed severely swollen ventricles and extremely thin cortex. This day 20 rat was manifestly hydrocephalic before necropsy. The response rate of one of the two mildly hydrocephalic animals was within control ranges and that of the other was lower than controls. No other significant gross or microscopic lesions appeared in the brains of the adult animals.

Brain, liver, and kidney concentrations of cadmium were evaluated in the adult animals after all behavioral tests were completed (Fig. 5). Figure 5 also includes liver and kidney concentrations of postweanling hydrocephalic rats exposed to 6 mg/kg but these values were not included in the statistical analysis because they came from younger rats.

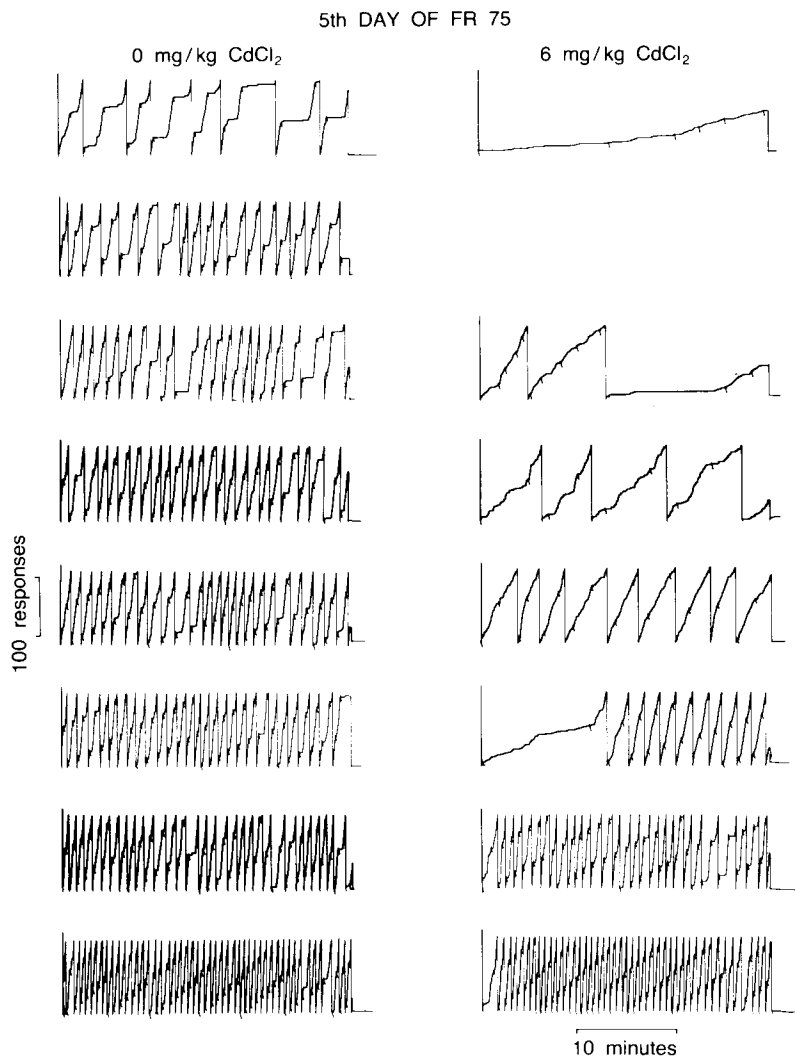


Fig. 3. Cumulative records of 0-mg/kg rats (N = 8) and 6-mg/kg rats (N = 7) on day 5 of FR 75 responding. The response pen advanced for each response and deflected downward for each reinforcer. The pen reset after 140 responses. The records are arranged in increasing rate from top to bottom with the lowest rate appearing on the top. Note that the control rats' performances can

be described as a pause followed by a high rate of responding. Four of the 6-mg/kg rats displayed erratic performance at this stage of training. The top record separated from the others represents a rat whose performance prevented a shift to an FR 75. The second rat in that column was shifted to an FR 15 after 3 days of FR 75 and maintained at that level.

A significant interaction between tissue and dosage appeared ($F(6,134) = 2.86, p = 0.0117$) as well as a main effect of both tissue ($F(2,134) = 50.8, p < 0.0001$) and dosage ($F(3,134) = 103.4, p < 0.0001$). Newman-Keuls multiple comparisons revealed that kidney and liver concentrations of cadmium were the same at each dosage and that both were significantly higher than brain concentrations. For both liver and kidney, the cad-

mium concentrations at dosages of 0, 1, 3, and 6 mg/kg was as follows: $0 < 1 < 3 = 6$. Although not included in the statistical tests, it appears from Figure 4 that the 22-day-old animals with frank hydrocephalus had higher kidney and liver cadmium concentrations than the adults exposed to the same dosage. The brain cadmium concentration in the 6-mg/kg animals was higher than in controls or the 1-mg/kg animals and that for the

TRANSVERSE SECTIONS OF 3 RAT BRAINS AT THE LEVEL
OF THE OPTIC CHIASMA (40 ×)

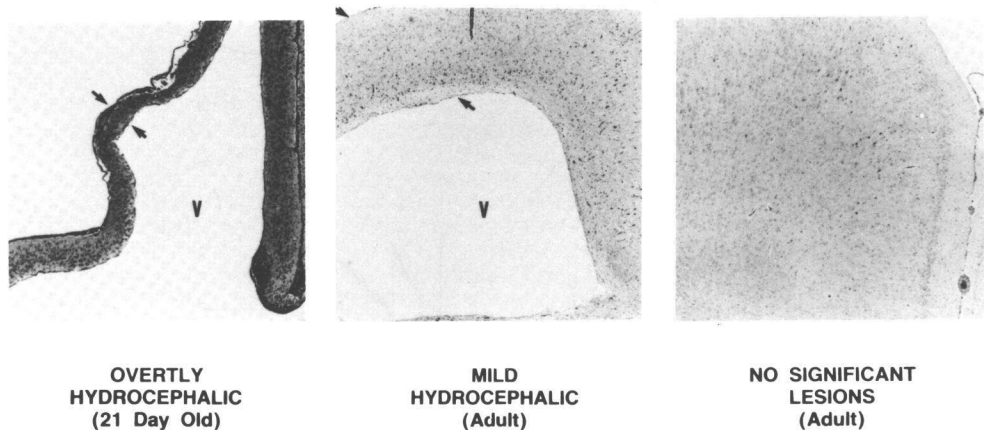


Fig. 4. Transverse section from three brains at the level of the optic chiasma showing examples of frank hydrocephalus (left), mild hydrocephalus (center), and no significant lesions (right). All magnifications are identical (40×). The median fissure is on the right edge of each photomicrograph. On the left two photomicrographs, the cortex is indicated with opposed arrows and the ventricle with V. The cortex of the normal adult was so thick it

filled the entire view. Note the extremely thin cortex and enlarged ventricle on the brain rated as severely hydrocephalic. This animal displayed retarded weight gain and an enlarged head prior to necropsy and was sacrificed at 21 days of age because of his condition. The case of mild hydrocephalus was not detected until histopathological examination. The cortex of the control animal was thick and ventricles do not appear in this field.

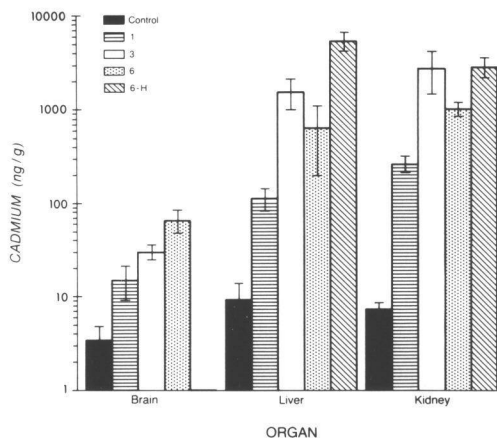


Fig. 5. Cadmium concentration (mean \pm 1 SEM) in the brain, liver, and kidney of the adult rats receiving 0, 1, 3, and 6 mg/kg, CdCl₂ at 1 day of age (first four bars in each group). The fifth bar in the kidney and liver groups represents hydrocephalic animals killed when they were 22 days old.

3-mg/kg animals was intermediate between the 1- and 6-mg/kg animals though not significantly different from either.

Concentrations of cadmium in different tissues were highly correlated: the correlation between brain and liver was 0.85, between brain and kidney was 0.78, and between liver

and kidney was 0.87. No relationship appeared between concentration in any tissue and the number of responses during the transition from FR 25 to FR 75.

Tissue concentrations were also obtained from a small group of rats injected with 10 mg/kg of cadmium on PN 1, but which died before PN 10. The average concentrations were 42,900 ng/g for liver (N = 5, range = 3400–79,000 ng/g), 8,670 ng/g for kidney (n = 3, range = 5,140–13,020) and 284 ng/g for brain (N = 1).

DISCUSSION

Behavioral effects

The present findings demonstrate enduring behavioral effects of acute neonatal exposure to cadmium chloride. The earliest adult behavioral effects of cadmium appeared during training; those animals exposed to 6 mg/kg CdCl₂ were hyperresponsive to the noise of the dipper and were difficult to train. The early differences did not persist; after training these animals resembled controls.

A striking and more persistent effect followed the shift to the FR75 schedule; this was the appearance of substantial increases in response output at 3 mg/kg and decreases at 6 mg/kg. The rate decreases reflected dif-

ferences between control and high-dose animals in the pattern of responding. After 5 days on the FR75 contingency the response pattern typical of fixed-ratio performance emerged in control animals. In contrast, the animals exposed to 6 mg/kg CdCl₂ continued to respond erratically.

The changes in responding persisted for several sessions following the transition to the highest fixed ratio and some rats never returned to control values. The behavioral effects appeared most strongly during the transition from FR 25 to FR 75, but some differences in the acquisition of lever pressing and steady-state fixed-ratio performance also appeared. The inverted U relationship between response rate and dose, showing elevated rates at moderate doses and low rates at high doses, is not unique to cadmium. Such a relationship has also been demonstrated with chronic exposure to lead (Cory-Slechta and Thompson, '79; Cory-Slechta et al., '83), as well as many drugs (Seiden and Dykstra, '77). The sensitivity of transition states has also been reported with lead (Mele et al., '84) and many drugs (Thompson and Moerschbaecher, '79).

In earlier reports (Newland et al., '83; Ng et al., '84) the animals exposed to 6 mg/kg fell into two groups, one with and another without hydrocephalus. Associated with frank hydrocephalus at PN 21 were performance deficits on preweaning tests of suckling, preference for home bedding, and neurological function. Animals without hydrocephalus, however, resembled controls on these preweaning evaluations. Hydrocephalus was detected only upon postmortem examination in two of the high-dose animals in the present report. One of these animals resembled controls on all earlier evaluations; the other had difficulty with the homing and suckling tests, but resembled controls on other functional evaluations. Overall, the animals exposed to 6 mg/kg CdCl₂ in the present report appeared normal on preweaning evaluations, but began to differ from control animals after PN 60, when growth was retarded in three animals.

The appearance of persisting effects on operant behavior in animals lacking morphological abnormalities stands in contrast to the earlier preweaning evaluations, which revealed behavioral aberrations only in pups with frank hydrocephalus. This finding demonstrates both the usefulness of schedule-controlled behavior for detecting subtle ef-

fects of substances (Weiss, '83) and the importance of extending evaluations of behavioral teratology into adulthood (Adams and Buelke-Sam, '81).

It should be pointed out that a cohort of the 3- and 6-mg/kg animals contained rats with cerebral hemorrhages on the day after exposure, but all evidence of hemorrhage disappeared after a few days (Newland et al., '83). It was impossible, of course, to determine the relationship between early hemorrhaging and later measures. The adults that had been exposed to 3 mg/kg resembled controls in gross appearance and body weight, and showed neither gross nor microscopic brain lesions upon post-mortem examination.

The differences between the preweaning and adult evaluations may reflect age differences in the expression of toxicity or the different types of behavior examined. Operant behavior represents a class of behavior different from that examined on preweaning evaluations. An operant like lever pressing is under the powerful control of the consequences of responding as shown by changes in the pattern and form of operants when environmental contingencies change (Fester and Skinner, '57). Aberrant changes in operant behavior in response to shifts in environmental contingencies can represent an important functional deficiency (Weiss, '83).

The low response rates of the 6-mg/kg group do not seem to be related to body weight differences or inadequate deprivation, but a different experimental design is required to rule out these effects conclusively. The low rates may be related to the extreme reaction of these animals, early in training, to the noise of the dipper. This suggestion corresponds with the finding of Nation et al. ('83) that cadmium increased the shock-induced suppression of responding, i.e., that cadmium treatment made animals more reactive.

The d-amphetamine sessions offered a pharmacological challenge that contrasts with the behavioral challenges imposed by the changing contingencies. The effects were typical of d-amphetamine's effect on FR performance: A slight overall rate increase appeared at low doses and low rates appeared at high doses (cf. Dews and Wenger, '77). That there was no interaction between cadmium and d-amphetamine is evidence that the deficits observed were specific to the behavioral challenges imposed. Rather than being nonspecific effects of any challenge,

the effects of cadmium depended upon the demands placed upon the subject.

d-Amphetamine was selected for the pharmacological challenge because of a previous report that cadmium might interact with apomorphine, a catecholamine agonist (Smith et al., '82). The lack of an interaction between d-amphetamine, a catecholamine agonist (Cooper et al., '82), and cadmium provides behavioral support for other findings that there are no long-term interactions of cadmium with catecholamine neurotransmitter systems (Rastogi et al., '77; Singhal et al., '76). Further work examining neuropharmacological correlates of cadmium toxicity should investigate drugs that act on cholinergic or serotonergic transmitter systems because of the potential involvement of these systems (Cooper and Manalis, '84; Singhal et al., '76).

Tissue concentrations

The elevated tissue levels of cadmium replicate and extend the findings of others that neonates exposed to cadmium have high brain, liver, and kidney levels. They extend these findings by demonstrating that cadmium persists in these organs, including the brain, after a single neonatal exposure and therefore correspond with reports that cadmium has a very long biological half-life (Ryan et al., '82). The lack of a monotonic dose-response relationship in the adult probably indicates that the cadmium concentrations in the adult animals represent the maximum amount tolerated by rats, i.e., higher tissue concentrations are fatal. This speculation is supported by the finding that the hydrocephalic animals and animals exposed to 10 mg/kg of cadmium chloride had substantially higher concentrations than those that survived. A direct comparison is difficult, however, because of age differences.

The persistence of cadmium in the brain after a single exposure is of particular interest, because it suggests a potential for long-lasting neurotoxicity resulting from acute exposure. Exposure to cadmium in a lipid-soluble form or before the blood-brain barrier is fully developed can result in elevated brain levels of cadmium (Gale et al., '82; Klaassen and Wong, '82). Thus chronic, direct neural effects of cadmium are possible even after a single exposure if the conditions favor entry into the central nervous system.

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