

# Behavior in Adulthood and During Aging Is Affected by Contaminant Exposure in Utero

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## Abstract

Environmental contaminants can alter the course of neural development, with consequences that appear in behavior. Such effects extend into adulthood and sometimes accelerate the rate of aging, even when exposure ceases by birth. The neurotoxicant methylmercury provides an interesting case study that reveals much about how disrupted neural development has lifelong consequences. Methylmercury also provides an example of the assessment and management of risks associated with exposure to developmental neurotoxicants.

## Keywords

methylmercury; behavioral toxicology; delayed neurotoxicity; development and aging

In April of 1956, two sisters entered the pediatrics department of a hospital in southern Japan. Previously bright, verbal, and active, suddenly they could not walk, their speech was incoherent, and they were delirious. Eventually a number of children from the same neighborhood entered the hospital with nearly identical complaints (Smith & Smith, 1975).

This was the beginning of a major industrial disaster caused by tons of mercury that were being dumped into Minamata Bay. Adults became blind, and children were born with

cerebral palsy and mental retardation. By 1993, 2,256 children and adults were diagnosed with Minamata disease in the fishing village that gave methylmercury poisoning its name (Harada, 1995). Methylmercury-contaminated fish were identified as the cause of the disease only after an all-too-familiar practice of blaming the victims for negligence, sinfulness, or drug abuse (Smith & Smith, 1975). (The pattern by which this and other disasters often unfold is captured closely in the fictional allegory *The Plague*, by Camus, 1947/1972.) The events in Minamata led researchers to recognize that developmental disorders can have environmental sources. Now these events are showing that disorders associated with aging may be related to contamination, too. Beginning at about 50 years of age, Minamata residents exposed to methylmercury as adults reported difficulties with such simple activities as buttoning a shirt or toileting themselves without assistance (Kinjo, Higashi, Nakano, Sakamoto, & Sakai, 1993), and this decline in function accelerated with age. Interestingly, death rates in exposed populations were no different from those in nearby villages, so the functional deficits are not necessarily linked to mortality.

In this article, we examine methylmercury neurotoxicity as a case study to illustrate the role that environmental contaminants can play over the course of a life span. We also hope to show how controlled studies can shed light on neural

and behavioral mechanisms by which methylmercury has its effects. Such understanding can inform the development of guidelines regarding exposure to neurotoxic substances.

## LABORATORY MODELS OF MINAMATA DISEASE

With methylmercury, as with many other chemicals, epidemiological evidence from human populations is correlational and cannot demonstrate causality or identify mechanisms of action. There are simply too many confounding influences. Controlled experimental studies with animals are necessary, especially with neurotoxic substances, because the effects are often irreversible and deliberate human exposure would be reprehensible.

Laboratory investigators have used nonhuman primates and rodents to study methylmercury's toxicity. Investigations with nonhuman primates reproduced the essential features of methylmercury exposure during neural development, resulting in a far better understanding of visual, auditory, and sensorimotor deficits associated with methylmercury and the exposure conditions required to produce them (Rice, 1996). Some primate studies also yielded intriguing evidence that animals that were exposed developmentally and appeared normal as adults showed deficits as they aged, but the sample sizes of these studies were small. Rodent studies had larger sample sizes but usually were less revealing about methylmercury's neurotoxicity. Sometimes no effects could be identified, or they occurred only at very high exposure levels. This discrepancy between primates and rodents sometimes led to suggestions that rats and mice are inappropriate models of human neurotoxicity of methylmercury (and sometimes of other chemicals as well).

We disagree. The difficulties with rodent studies were related to dosing regimens and the behavioral measures employed. Primate studies entailed chronic, low-level exposure regimens and sophisticated behavioral procedures designed to identify subtle effects of exposure. Rodent studies often incorporated acute, high-level exposure that could only result in wildly changing methylmercury concentrations in the brain, so the methylmercury concentration at certain crucial developmental periods could not be ascertained. Sometimes these studies used behavioral measures that are better suited to demonstrating the effects of high exposure levels than to identifying subtle impairments associated with chronic, low-level exposure (Newland & Paletz, 2000).

With appropriate experimental design, the rodent can be an excellent model of human mercury exposure, however. Stable mercury concentrations comparable to those seen in primate studies can be produced in the rodent brain by adjusting methylmercury intake to overcome the exceptionally high levels of mercury-binding hemoglobin in rat blood (because mercury binds readily to sulfur, found in hemoglobin, relatively less mercury is available for transport to the brain in rats than in other mammalian species) and by beginning exposure weeks before mating, to allow for the long time required for methylmercury levels to stabilize. In our studies (Newland & Reile, 1999), female rats consume water containing 0, 0.5, or 6 ppm of mercury (as methylmercury), resulting in intakes averaging about 0, 40, and 500  $\mu\text{g}/\text{kg}/\text{day}$ , respectively, before mating. The resulting brain concentrations (about 0, 0.5, and 9 ppm) are in a range considered to be low to moderate (Burbacher, Rodier, & Rice, 1990) in mammalian species. These levels are quite stable because of the protracted

dosing regimen. Our observations confirm that these are not high exposure levels. It would be impossible to identify rats exposed under our protocol using cage-side observations, even if one were looking specifically for methylmercury-related signs.

#### PUZZLING ABOUT LEARNING AND MEMORY

Epidemiological studies have correlated methylmercury exposure with mental retardation at high exposure levels (Harada, 1995) and with subtle changes in language and attention tasks at lower levels (Grandjean, Weihe, White, & Debes, 1998). Paradoxically, methylmercury exposure in rats or monkeys has not affected performance on tasks commonly associated with "cognition." Performance is not impaired on discrimination tasks in which one stimulus (an S+) signals the availability of reinforcement and another (an S-) signals that reinforcement is not available. Nor do effects appear after the S+ and S- are reversed and a new discrimination must be acquired. Memory is not impaired either; methylmercury may even have increased delays at which monkeys' performance on a delayed discrimination task began to deteriorate (reviewed in Newland & Paletz, 2000, and Rice, 1996). These tasks emphasize the contextual control of behavior, and they appear to be insensitive to methylmercury. A procedure that emphasizes the selection of behavior by reinforcing events, described shortly, appears to be quite sensitive to methylmercury. A crucial distinction exists here, and it draws from the insight that operant behavior (essentially all voluntary behavior) can be understood by reducing it to a three-term contingency of reinforcement, in which a response-

reinforcer relationship is viewed as acting in a stimulus context. First noted more than half a century ago by Skinner, the ability of this dynamic interplay among stimuli, responses, and consequences to account for the formation of impressively complex behavior is among the most widely replicated empirical phenomena in all of psychology.

Experiments can be designed to examine different terms, even if these terms cannot be completely isolated. In a discrimination task, the experimenter changes something about the stimuli that signal which response to perform. Thus, a red light may signal that pressing the left lever produces food, and a green light may signal that pressing the right lever produces food. In a memory task, the stimuli are removed before the opportunity to respond is made available. The response-reinforcer relationship is invariant—one lever press always produces food, for example. Accuracy is often used to measure contextual control over behavior (synonyms include *stimulus control* or *discrimination*).

To emphasize the response-reinforcer relationship, researchers hold the stimulus context constant, but the relative rate of reinforcement available from different response devices changes. In this case, behavior change has little to do with context because that remains constant. Instead, behavior change reflects different response-reinforcer relationships. For example, two levers may both produce food twice a minute for a few sessions, but then one lever produces food four times per minute and the other produces food once per minute. Context still exists (the levers are, after all, different), but its role is deemphasized relative to the role played by the specific response requirements. This thinking has been applied to methylmercury's neurotoxicity as follows (Newland & Paletz, 2000).

Figure 1 illustrates the effect of in utero methylmercury exposure on behavior of squirrel monkeys in a procedure that examines continuous choice under reinforcement contingencies that change occasionally (Newland, Yezhou, Logdberg, & Berlin, 1994). In this experiment, a squirrel monkey faced a panel containing two levers. In the first phase, pressing the left lever produced food intermittently but unpredictably once a minute. Pressing the right lever produced food under the same schedule of reinforcement. By switching between the two levers, the monkey could receive an average of two reinforcers per

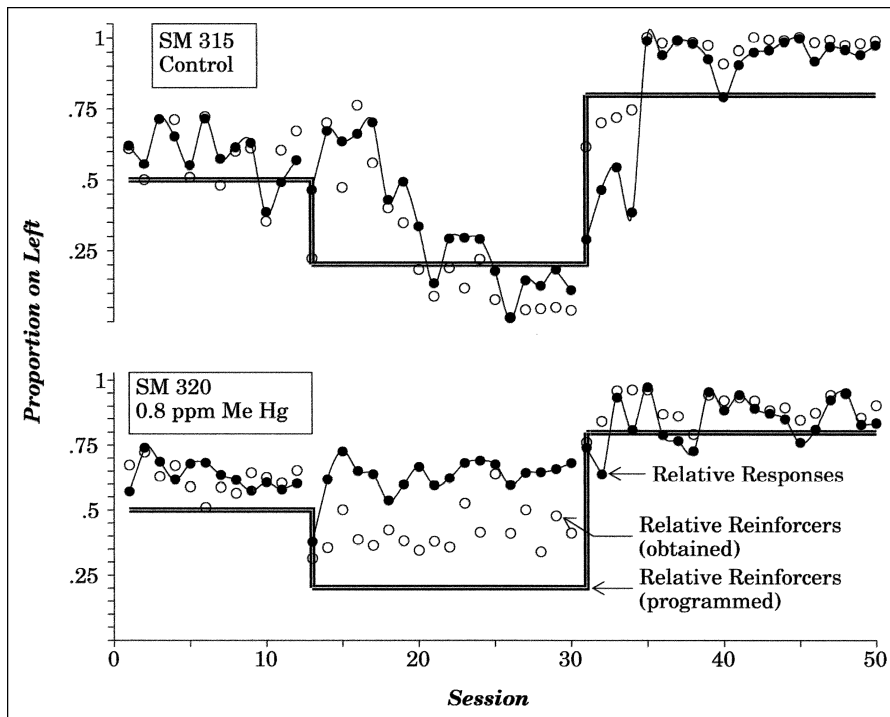
minute. (This is called a concurrent schedule of food reinforcement.)

Reinforcement rates changed abruptly at the beginning of the 13th session; the left lever produced food at one quarter the rate of the right lever (one vs. four reinforcers per minute). The behavior of the control monkey, which was not exposed to methylmercury, gradually shifted until the proportion of its responses on the left lever approximated the low proportion of reinforcers delivered by that lever. This steady-state performance in which the relative allocation of behavior approximates the relative availability of reinforce-

ment is called matching and is commonly observed in studies of animals (Davison & McCarthy, 1988) and humans (Kollins, Newland, & Critchfield, 1997).

In contrast, the behavior of the methylmercury-exposed monkey was unperturbed by the new reinforcement rates. This insensitivity also occurred in later transitions, including the one beginning at Session 31 in Figure 1. In behavior therapy interventions (results not shown in the figure), 99% of the reinforcers were programmed to come from one lever, an extreme discrepancy that finally caused exposed monkeys' behavior to change. Three methylmercury-exposed monkeys, and many lead-exposed monkeys, exhibited retarded transitions repeatedly with this procedure; that is, they required many more reinforcers to complete the transition than did unexposed monkeys. These results have been replicated with rodents in as-yet unpublished data.

In this type of study, it is common practice to correlate the relative number of responses on a lever to the relative reinforcement rate obtained from that lever. However, the monkey study just described (Newland et al., 1994) emphasized programmed reinforcement rates instead. An extreme example exemplifies the difference between programmed and obtained reinforcement rates. The experimenter may arrange for (program) one quarter of the reinforcers to derive from the left lever, but if no responses occur on that lever, then 0% of reinforcers are obtained from that lever. Programmed reinforcement rate is a more appropriate variable because (a) the goal is to examine how neurotoxicants alter the way in which structure in behavior reflects structure in the environment and (b) obtained reinforcement proportions are not independent variables, anyway. To see the latter point, note that during the first transition for the methylmercury-exposed monkey (Fig.



**Fig. 1.** Lever pressing of 2 squirrel monkeys undergoing transitions in concurrent reinforcement schedules. Initially, one half of the food reinforcers derived from the left lever and one half derived from the right lever. Beginning with the 13th session, only 20% of the reinforcers derived from the left lever, and beginning in the 31st session, 80% of the reinforcers derived from the left lever. Each datum represents a single, 30-min session. The double lines show the proportion of reinforcers programmed to derive from the left lever. Open circles show the proportion of reinforcers obtained from that lever. Closed circles show the proportion of responses made on that lever. Results are shown for a control monkey (top panel) and a monkey exposed in utero to methylmercury (Me Hg; bottom panel). Adapted from "Prolonged Behavioral Effects of in Utero Exposure to Lead or Methyl Mercury: Reduced Sensitivity to Changes in Reinforcement Contingencies During Behavioral Transitions and in Steady State," by M.C. Newland, S. Yezhou, B. Logdberg, & M. Berlin, 1994, *Toxicology and Applied Pharmacology*, 126, p. 8. Copyright 1994 by Academic Press.

1), obtained reinforcement rates lay between programmed rates and response proportions. Obtained reinforcement rates depended on both programmed reinforcement rate and behavior and cannot be considered independent.

### METHYLMERCURY EXPOSURE IN UTERO INFLUENCES THE COURSE OF AGING

We now shift from young monkeys to old rats. We followed rats exposed to methylmercury during gestation (as described earlier) throughout life to examine very long-term consequences of such exposure (Newland & Rasmussen, 2000). When the rats were 4 to 6 months of age, we trained them to press a lever nine times within 4 s. (This is referred to as differential reinforcement of high rate, meaning that high response rates, including those that make up response bursts, are selectively reinforced with food.) We focused on the number of nine-response bursts that met the high-response-rate criterion, as well as on the age at which performance declined to 50% of the levels seen when the rats were young adults.

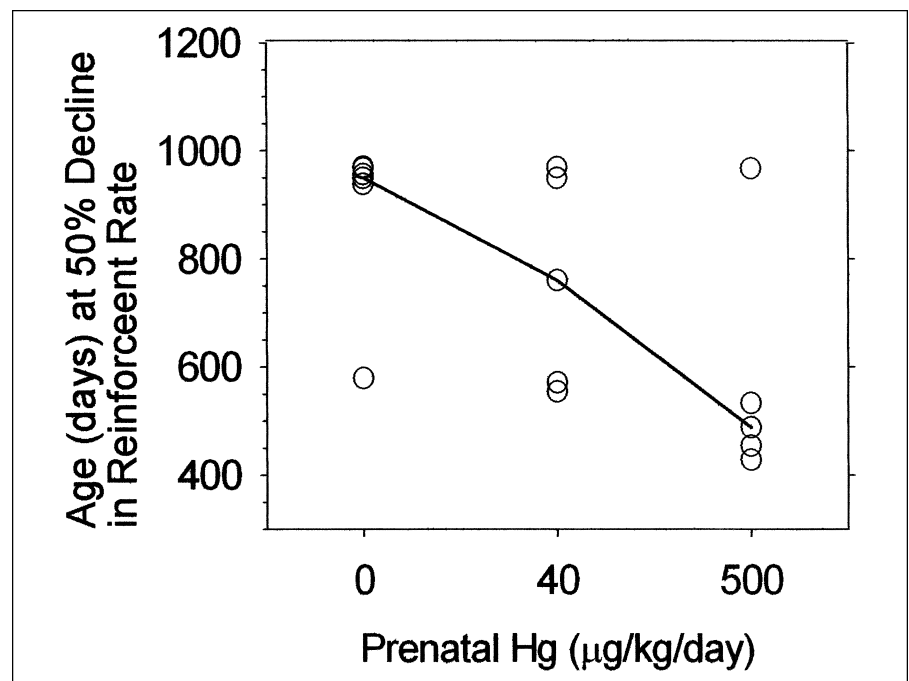
Aging exerted its own toll on this behavior. Most 2-year-old control rats performed at about 80% of the baseline established when they were young adults, but 1 crossed the 50% threshold (Fig. 2). As the figure illustrates, in utero exposure to methylmercury caused many rats to cross the threshold at a younger age, and the higher the exposure, the younger the age. The exposed animals had not had methylmercury since weaning and probably not since birth because little methylmercury is available in milk (Newland & Reile, 1999). Figure 2 also illustrates the phenomenon known as individual susceptibility, a common finding in behavioral toxicology. In each exposure group, at least 1 rat com-

pleted the study without crossing the 50% threshold, and at least 1 rat crossed this threshold; the number showing performance deficits increased with methylmercury dose. Thus, it is not the case that in utero methylmercury exposure shifted the whole population equally. Instead, it appears that some rats are susceptible to showing functional declines as they age, and methylmercury exposure in utero amplified this susceptibility. Incidentally, mortality in these rats was unrelated to exposure, as in Minamata.

### DRUG CHALLENGES AND NEUROCHEMICAL MECHANISMS

Can a behavioral mechanism such as reinforcement insensitivity be re-

lated to neurotransmitter function? By observing a contaminant's effects in vitro (i.e., on isolated neural tissue), researchers form hypotheses about its neurochemical mechanisms of action. To be meaningful, these hypotheses must be tested with drug challenges, in which a drug with known, specific effects is administered to animals engaging in a behavioral task. Early, and somewhat limited, studies suggested that exposure to methylmercury during development increases sensitivity to *d* amphetamine, a drug that promotes the activity of dopamine and norepinephrine neurotransmitter systems. We expanded on these studies by including drugs representing several drug classes, selected according to their effects on tissue; by examining a full range of doses; and by using fully adult animals (Rasmussen & Newland, 2001).



**Fig. 2.** Decline in reinforcement rates of rats trained to execute response bursts of nine lever presses within 4 s in order to receive food reinforcement. The graph shows the age at which each rat's performance (number of bursts meeting the criterion) declined to 50% of a baseline established when the rat was a young adult. If the rat completed the experiment without experiencing such a decline, a value of 950 days was entered. Results are shown for rats exposed to methylmercury in utero and control rats with no exposure. Adapted from "Aging Unmasks Adverse Effects of Gestational Exposure to Methylmercury in Rats," by M.C. Newland & E.B. Rasmussen, 2000, *Neurotoxicology and Teratology*, 22, p. 825. Copyright 2000 by Elsevier Science, Inc.

We conducted these drug challenges with the same rats described in the previous section; their lever pressing was maintained under the same differential-reinforcement-of-high-rate schedule of reinforcement as in that study. The drug challenges were conducted when the rats were between 6 months and 1 year old. To determine a drug's effect, we injected it before an experimental session and compared the resulting response rates with those seen in control sessions in which either there was no injection or an inert solution was injected. Multiple doses were used. To rule out non-specific effects of the injection process itself, we occasionally injected only the vehicle, the fluid in which the drug was dissolved. Compared with unexposed rats, methylmercury-exposed rats were up to twice as sensitive to *d* amphetamine; that is, the dose that significantly lowered responding in the methylmercury-exposed rats was one half the dose with the same effect in the unexposed rats. Exposed rats were less sensitive to pentobarbital, which promotes the activity of an inhibitory transmitter called GABA (gamma amino butyric acid). Equally important is the fact that the study demonstrated specificity: Methylmercury-exposed rats showed no differential sensitivity to other drugs tested.

There is substantial evidence that midbrain dopamine, and perhaps norepinephrine, pathways play a role in reinforcement and choice. The methylmercury-exposed rats' sensitivity to amphetamine might indicate that their diminished sensitivity to reinforcing consequences, illustrated in Figure 1, is related to actions of these neurotransmitter systems. In other words, it appears that a behavioral effect of methylmercury exposure, sensitivity to reinforcement, can be linked to a specific neural mechanism, alteration in the dopamine system. Many gaps remain to be filled, including

relating *d* amphetamine's behavioral effects to altered behavioral transitions (illustrated in Fig. 1) before this can be verified.

In a similar vein, pentobarbital's actions might be viewed in light of observations that compounds that promote GABA, like alcohol, pentobarbital, and many tranquilizers, can cause selective amnesia. The diminished sensitivity to pentobarbital associated with methylmercury exposure might be related to reports that methylmercury does not disrupt performance on tasks that tax memory. At present, however, this idea is only speculation.

#### LINKING ANIMAL AND HUMAN EXPOSURES TO ASSESS RISK

The episode at Minamata showed not only that methylmercury is a hazard, but also that it is found in fish. It is now known that fish is the major source, close to the only source, of human methylmercury exposure worldwide. Therefore, advice about consuming methylmercury will influence the consumption of fish, an excellent source of nutrients important to neural development and cardiovascular health. It is crucial to understand how methylmercury acts, and at what doses, to ensure that advisories are not drawn so cautiously that they reduce fish consumption inappropriately. Laboratory studies are a necessary component of the process of identifying acceptable exposure levels. In our studies, and in some others, the duration, magnitude, and route of exposure were selected after considering the biology underlying methylmercury intake and elimination. However, the studies also model human exposures and therefore can contribute to evaluations of risk. Effects that are dose related, reproducible, and linked to mecha-

nisms of action can be combined with epidemiological studies to arrive at estimates of a *reference dose*, a level of intake that is unlikely to be harmful.

Creativity and skillful application of principles of conditioning in designing behavioral procedures are key to identifying the subtle effects of low-level exposure, and the effects of low doses receive considerable attention in policymaking regarding the even lower exposure levels that people might experience. Even under the best of circumstances, it is necessary to extrapolate to doses lower than those used in laboratories. Studies with economically feasible sample sizes will not detect effects seen in fewer than 10% of subjects, but a 10% prevalence would be a disaster in a human population. The solution to this problem is beyond the scope of this article, but readers might be interested in seeing the creative approaches taken to conducting such extrapolations (Glowa & MacPhail, 1995). These approaches exploit the quantitative sophistication of well-designed behavioral experiments.

After reviewing the scientific literature, the U.S. Environmental Protection Agency (EPA) recently set the reference ("safe") dose for methylmercury at 0.1  $\mu\text{g}/\text{kg}/\text{day}$ , or about one can of tuna per week, for pregnant women. The fetus was the primary concern because it was felt to be the most sensitive to methylmercury's effects. Effects associated with aging might extend concern to the elderly.

The value of 40  $\mu\text{g}/\text{kg}/\text{day}$  that caused impairments in our rats might seem far removed from the reference dose, but in light of how risk assessment is actually conducted, it may be quite close. Risk assessors acknowledge that there are uncertainties embedded in extrapolating from small, relatively normal, and otherwise healthy rats to a diverse array of people. To ac-

commodate these uncertainties, they simply divide the exposure level used in a laboratory study by one or several powers of 10 to estimate tolerable human exposures. The point of departure for conducting a risk assessment beginning with rats might be 4 and not 40  $\mu\text{g}/\text{kg}/\text{day}$ , because of the high concentration of hemoglobin in rat blood, but it is not clear how to incorporate this peculiarity of rat blood into risk assessment. Thus, the intake experienced by our animals could be uncomfortably close, by risk-assessment standards, to the level considered unlikely to cause harm in humans.

EPA's reference dose aroused considerable debate because of how it might influence fish consumption. Setting reference doses too low or communicating a message so confusing that it dissuades people from eating fish would be counterproductive. As a state risk assessor told us, "If I tell people to avoid *certain* fish, then they will simply avoid *all* fish and eat burgers and fries instead!"

Fish differ widely in mercury content and in nutrients. For example, swordfish and shark may contain 10 to 40 times the mercury found in tuna, and ocean salmon may have 10 times less. So only large, long-lived predators (mercury accumulates in the food chain and in long-lived fish) should be avoided. Health agencies recommend avoiding shark, king mackerel, tilefish ("golden bass," "golden snapper"), swordfish, and fish from contaminated waters, but these recommendations are confusing and widely ignored. Swordfish is found on many restaurant menus, and few people know what is contained in processed fish. Perhaps people would be better served if hazards were simply removed from the food supply, so consumers could be assured that the fish they do purchase will not cause harm.

## CLOSING COMMENTS

Psychology can make a significant contribution to the environmental health sciences. The experimental analysis of behavior, by applying well-grounded principles of conditioning, already has. Experimental psychology has more than a century's experience in grappling with the difficult problem of studying behavior systematically. This experience has yielded many successes in identifying fundamental behavioral principles and in linking these to nervous system activity. In addition, experimentalists have developed many creative methods for examining behavior in exquisite detail. Clinical psychology, when it draws from science, can contribute to treatment, assessment, and the application of principles. All of this expertise can be used in ways that matter to science and to policy.

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## Note

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