Age-Related Increase in Auditory Impairment in Monkeys Exposed in Utero plus Postnatally to Methylmercury

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Hearing deficits are a frequent result of methylmercury poisoning in adults (Al-Damluji et al., 1976; Harada, 1968, 1977). Assessment of pure-tone detection thresholds, tested in the range of speech frequencies (0.5–2 kHz), revealed hearing impairment in half the ears tested (Ino and Mizukoshi, 1977). Severe hearing impairment, deafness, and delayed speech development have been reported as a result of in utero exposure to methylmercury (Marsh et al., 1980; Amin-Zaki et al., 1974, 1979; Brenner and Snyder, 1980). However, the methodology used to assess hearing in these studies was unspecified, and results of assessments were not provided. More recently, a prospective study in a fish-eating population in the Seychelles Islands reported deficits in auditory comprehension in children with low exposure to methylmercury (Myers et al., 1995). Since auditory function per se was not assessed, it is not known whether this finding represents sensory impairment or some other nervous system deficit.

In humans, developmental exposure to methylmercury produces a pattern of neuropathology different from that observed in adults (Takeuchi and Eto, 1975; Takeuchi, 1968). In adults, deep sulci are preferentially damaged, while in utero exposure results in damage that is much more uniform throughout the brain. Infantile exposure results in a pattern of damage that is intermediate between these patterns. The adult macaque monkey exhibits central nervous system pathology similar to that of adult humans after exposure to methylmercury (Evans et al., 1977; Shaw et al., 1975; Garman et al., 1975). While the auditory cortex has not been specifically examined after methylmercury exposure, temporal cortical damage has been observed in both monkeys (Garman et al., 1975) and humans (Takeuchi et al., 1962). Primary auditory cortex lies within the fissure of the superior temporal gyrus and is thus a good candidate for preferential damage by methylmercury following adult or infantile exposure (Takeuchi and Eto, 1975). In utero exposure may be expected to produce diffuse damage to auditory cortex.

There is evidence for delayed or accelerated neurotoxicity during aging as a consequence of previous methylmercury exposure (Rice, 1996). In an important study including more than 90% of patients diagnosed with Minamata disease (MD) 20–30 years previously, it was reported that the ability to independently eat, bathe, dress, wash the face, and use the toilet decreased in an age-related manner in MD patients compared with age-matched controls (Kinjo et al., 1993). In other words, there was an interaction between aging and previous exposure to methylmercury. In a long-term follow-up study in persons previously diagnosed with MD, it was reported that auditory function had worsened (Harada, 1995). A “chronic” MD was also identified that had a different pattern of signs than early-onset MD and was characterized by a high incidence of somatosensory impairment (glove and stocking hypoesthesia) and auditory impairment. Harada (1995, p. 14) differentiated three patterns of worsening of signs of MD: “gradually pro-
gressive type, delayed onset type, and escalator progressive type with aging.” In our laboratory, we observed overt toxicity manifested as clumsiness at 13 years of age in a cohort of monkeys exposed to methylmercury from birth to 7 years (Rice, 1989a). These monkeys also developed additional signs of methylmercury-induced toxicity as they approached 20 years of age that had not been present when they were younger (Rice, 1996).

We previously reported high-frequency hearing loss in the cohort of monkeys dosed with methylmercury from birth to 7 years of age and tested at 14 years (Rice and Gilbert, 1992). The current study extends this research by assessing auditory function in monkeys exposed in utero to about 4 years of age to the same or lower doses as those in the previous study. Pure-tone detection thresholds were determined across most of the hearing range of macaque monkeys using a psychophysical (behavioral) procedure. All individuals were tested at 19 years of age, while some individuals were also tested at 11 years, allowing comparison of auditory function at middle age and during aging.

METHODS

Subjects. Methylmercury-exposed monkeys (Macaca fascicularis) were exposed in utero and continuing after birth until about the age of puberty. The mothers of the infants were dosed three times per week with the equivalent of 10, 25, or 50 µg/kg/day of mercury as methylmercuric chloride added to a small amount of juice. When at least 90% of the estimated blood equilibrium value based on a one-compartment model was reached (Rice, 1989a), females were bred to untreated males. Infants were separated from their mothers at birth and dosed with the same nominal dose their mothers had received, administered 5 days a week. Dosing was discontinued when offspring were 3.5–4.5 years of age. Five infants were born in the high dose, two at the intermediate dose, and one at the low dose. Two monkeys from the high-dose group were alive at 11 years of age when auditory function was first assessed, as were the three monkeys at the lower doses. Infants were born with blood mercury concentrations about 1.8 times higher than those of their mothers, which decreased with a half-life of 2–3 months for the monkeys in the current experiment to steady-state concentrations that were maintained for the duration of exposure. Modeling of blood mercury kinetics was carried out after termination of methylmercury exposure (Rice, 1989a), and included estimated concentration at birth and under steady-state conditions and the half-life of elimination following cessation of methylmercury exposure (Table 1). No monkey ever received any known ototoxic agent including aminoglycoside antibiotics. Overt signs of methylmercury toxicity such as nystagmus, obvious visual impairment, tremor, clumsiness, or ataxia were not observed in any monkey tested in this experiment.

Equipment and calibration procedure. Individual sine-wave frequencies were generated by a Krohn-Hite (Avon, MA) oscillator (Model 4141R). Sine-wave tones were fed through an attenuator to a rise-fall pulse shaper (Model 584-04, Coulbourn Instruments, Lehigh Valley, PA), audio-mixer amplifier (Coulbourn Model 882-24), and 40-db attenuator to the earphone (Sennheiser HD540 Reference Gold, Wedemark, Germany). Rise and fall time was a 200-ms linear ramp. The linearity and absolute value of frequency and amplitude were checked monthly. Headphone amplitude was calibrated using Bruel and Kjaer (Naerum, Denmark) precision sound level meter Model 4230, Bruel and Kjaer sound measuring amplifier 2610, Bruel and Kjaer artificial ear Model 4153, and Bruel and Kjaer 0.5-in. condenser microphones 4134 (to 20,000 Hz) and 4136 (to 40,000 Hz). The calibration data were used to produce a linear headphone response from 85 to 40,000 Hz.

<table>
<thead>
<tr>
<th>Dose (µg/kg/day)</th>
<th>Monkey No.</th>
<th>Sex</th>
<th>Birth</th>
<th>Steady state</th>
<th>l1/2 (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>101</td>
<td>F</td>
<td>2.00</td>
<td>0.80</td>
<td>12.7</td>
</tr>
<tr>
<td>25</td>
<td>102</td>
<td>M</td>
<td>2.70</td>
<td>0.78</td>
<td>14.9</td>
</tr>
<tr>
<td>10</td>
<td>104</td>
<td>M</td>
<td>0.45</td>
<td>0.22</td>
<td>13.6</td>
</tr>
<tr>
<td>0</td>
<td>117</td>
<td>M</td>
<td>nd</td>
<td>nd</td>
<td></td>
</tr>
<tr>
<td>117</td>
<td>118</td>
<td>F</td>
<td>0.80</td>
<td>0.41</td>
<td>9.4</td>
</tr>
<tr>
<td>116</td>
<td>118</td>
<td>F</td>
<td>0.80</td>
<td>0.41</td>
<td>9.4</td>
</tr>
</tbody>
</table>

* Not detected.

Behavioral procedure. Monkeys were initially tested beginning at 11 years of age, at which time complete data were obtained from treated monkeys 102, 116, and 118 and control monkeys 117 and 120. High-dose monkey 101 failed to generate reliable data after almost a year of training. Low-dose monkey 104 generated three reliable thresholds, at which time stereotypic behavior made further testing unusable. Monkey 101 subsequently easily learned a somatosensory task using the same psychophysical procedure as in the auditory experiment, and 104 also performed well on the somatosensory task with no behavioral problems. When monkeys were 19 years old, auditory thresholds were redetermined. Individuals who had generated complete data at 11 years of age were retested in the right only, while 101 and 104 were tested in both ears. The second assessment of all individuals was necessary for comparisons to be age-matched. Reassessment also provided the opportunity to compare possible degradation of auditory function over time in control and methylmercury-treated monkeys.

Schedule control and data acquisition were by means of a behavioral notation language (Gilbert and Rice, 1979) run on a Nova 4 minicomputer (Data General, Southboro MA). Data were collected as interevent times so that the session could be reconstructed from the raw data. The monkey sat in a primate chair, restrained at the neck and the waist, inside a sound-attenuating cubicle. Earpieces attached to the primate chair were precisely positioned over each ear for delivery of the auditory signal. Different-size chairs were used for individual monkeys as appropriate. The monkey initiated a trial by touching a stainless-steel bar which completed a ground loop sense by the computer. This resulted in a signal light facing the monkey changing from red to green and initiated a variable foreperiod of 2, 3, 5, or 7 s, chosen randomly within each group of four trials. After the foreperiod, the tone to be detected was delivered monaurally. The monkey’s response indicated detection or lack of detection of the stimulus (yes-no response paradigm). Release of the bar within 1.5 s resulted in offset of the tone and delivery of 0.5 ml of apple juice followed by a 3-s intertrial interval (ITI). Failure to release the bar within 1.5 s resulted in offset of the tone, the signal light changing from green to white, and initiation of a 10-s time-out (TO) period. Premature release of the bar before tone onset resulted in a TO period and a repeat of the trial. Sessions comprised 100 (11 years) or 30 (19 years) completed threshold testing trials. Thirty-three (11 years) or 16 (19 years) additional trials were catch trials with a 7-s foreperiod in which a tone 12 dB above that of the previous trial was used as the signal tone. Failure to release the bar within 5.0 s of the onset of the tone resulted in a TO period; release resulted in reinforcement. Monkeys were tested one session per day, 5 days per week. Monkeys were given a specified amount of water after each session, at least 50 ml/kg.

Only one ear and one frequency were tested within a session. No sound was delivered to the other ear. The method of stimulus presentation was the transformed up-down psychophysical method. The session began with a high-intensity sound that was above the threshold for that individual. Starting sound levels varied between 44 and 106 dB depending on the frequency being tested and auditory sensitivity of the individual. The first 15 correct responses
threshold at 1 kHz while thresholds for most other frequencies were lower than at 11 years.

High-dose monkey 101 had extremely elevated thresholds in both ears at all frequencies at 19 years of age (Fig. 2). The shape of her curve was also abnormal, with thresholds generally decreasing slightly as a function of increasing frequency. High-dose monkey 102's thresholds were elevated in both ears compared with controls at all but the highest frequency at 11 years of age; at 19 years of age, the three higher frequencies were impaired compared with performance at 11 years. Middle-dose monkey 116 had an elevated threshold at 10 kHz in the left ear at 11 years, while the threshold at 25 kHz in the right ear was sufficiently elevated that 31.5 kHz was not tested. At 19 years of age, high frequencies in the right ear were even more elevated, compared with controls, than they had been at 11 years. For middle-dose monkey 118, thresholds in the right ear appeared normal at 11 years, while the left ear had elevated thresholds at the three lowest frequencies. When retested at 19 years of age, she exhibited elevated thresholds in the right ear compared with the control monkeys at all but the highest frequency. Low-dose monkey 104 exhibited elevated thresholds in both ears at 19 years of age at all frequencies but the highest, with the right ear being more impaired than the left. The three data points collected when he was 11 years of age, on the other hand, were within the range of control values.

Comparison of the differences in auditory function between the ages of 11 and 19 years provides some evidence for differential impairment as a function of aging between control and methylmercury-exposed monkeys (Table 2). Monkey 102 and, to a lesser extent, monkey 116 exhibited a selective increase in impairment at higher frequencies, a pattern not observed in controls. Monkey 118 had no increase in threshold at 31.5 kHz between 11 and 19 years, while thresholds at most other frequencies were relatively more elevated than those of controls. Similarly, 104 had apparently normal auditory function for the frequencies tested at 11 years, while thresholds in both ears were elevated compared with controls at 19 years, with a differential in the right ear of 40 dB for the frequency tested at both ages.

<table>
<thead>
<tr>
<th>Dose (µg/kg/day)</th>
<th>Monkey No.</th>
<th>Frequency (kHz)</th>
<th>&lt;dB difference (19-11 years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>102</td>
<td>0.125</td>
<td>50.0</td>
</tr>
<tr>
<td>25</td>
<td>116</td>
<td>1</td>
<td>-5.0</td>
</tr>
<tr>
<td>10</td>
<td>118</td>
<td>10</td>
<td>-20.0</td>
</tr>
<tr>
<td>5</td>
<td>117</td>
<td>25</td>
<td>-20.0</td>
</tr>
<tr>
<td>0</td>
<td>120</td>
<td>31.5</td>
<td>-20.0</td>
</tr>
</tbody>
</table>

RESULTS

There were no differences between control and treated monkeys in measures of schedule control. Median reaction times were 550-750 ms irrespective of foreperiod value.

During assessment at 11 years of age, the two control monkeys displayed psychophysical functions that are typical of macaque monkeys, as discussed previously (Rice and Gilbert, 1992) (Fig. 1). Thresholds were elevated at the lowest and highest frequencies, and generally flat in between. When retested at 19 years of age, control monkey 117 had slightly to moderately elevated thresholds in the right ear at all frequencies, ranging from about 3 to 17 dB (Fig. 1) (Table 2). In contrast, control monkey 120 exhibited an 8.5-dB elevation in each decreased the amplitude by 3.0 dB; subsequent correct responses decreased the amplitude by 3.0 dB with a probability of one-third. Each failure to respond to the tone increased the amplitude for the next (noncatch) trial by 3.0 dB. This schedule results in threshold being estimated at 75% correct responses.

A session was included for threshold determination if the monkey made a premature release in fewer than 10% of noncatch trials and made either a premature release or a failure to release on a total of fewer than 15% of catch trials. Threshold was calculated by determining each crossing point for the last 60 (11 years) or 30 (19 years) noncatch trials of a session and determining the median. A crossing point was defined as the mean decibel value between each pair of changes of direction in amplitude; i.e., between when amplitude began increasing as the result of an error and decreasing as the result of a correct response according to the specified schedule criteria, or vice versa. A graph of the amplitude across trials was also printed for each session. In almost all cases the crossover points for the trials included for threshold determination were characterized by a lack of slope, i.e., the threshold drifting neither up nor down. Whether the performance was stable was determined by visual inspection. Sessions that did not meet these criteria were excluded. A threshold for each frequency for each ear was calculated as the mean of the lowest three daily sessions that did not meet these criteria were excluded. A threshold for each ear appeared normal at 11 years, while the left ear had elevated thresholds at the three lowest frequencies. When retested at 19 years of age, she exhibited elevated thresholds in the right ear compared with the control monkeys at all but the highest frequency. Low-dose monkey 104 exhibited elevated thresholds in both ears at 19 years of age at all frequencies but the highest, with the right ear being more impaired than the left. The three data points collected when he was 11 years of age, on the other hand, were within the range of control values.
FIG. 2. Auditory threshold functions for five monkeys exposed to methylmercury throughout gestation to 4 years of age. Each panel is identified with the appropriate monkey number and dose group (µg/kg/day) in parentheses. Individuals in which complete functions were generated in both ears at 11 years of age were retested in the right ear only at 19 years. Monkey 104 completed two points in the left ear at 11 years and one point in the right ear at 10 kHz (displaced along the x axis for clarity). Thresholds presented as in Fig. 1.

DISCUSSION

The threshold curves of the control monkeys at 11 years of age are in agreement with data in macaques from other investigators (Stebbins and Rudy, 1978; Stebbins et al., 1966; Pfingst et al., 1978), being elevated at high and low frequencies and comprising a relatively flat function between 1 and 25 kHz. At 19 years, there was a tendency for the threshold at 1 kHz to be elevated relative to thresholds between 4 and 25 kHz. Normal monkeys can hear at least an octave higher than normal humans; below 8 kHz the detection levels of humans and macaques are identical (Owren et al., 1988).

When tested at 19 years of age, all five methylmercury-exposed monkeys exhibited elevated thresholds for pure tones compared with controls, in some cases 50 dB or more. There was also a tendency for the functions of the treated monkeys to be relatively flat as a result of relatively greater impairment across the range of middle frequencies. It is tempting to speculate that the extreme hearing impairment of high-dose monkey 101 was responsible for her apparent inability to learn the behavioral task necessary for auditory testing at 11 years. Exposure to the identical behavioral task used in the current study to assess somatosensory function enabled her to subsequently transfer that experience to the auditory task, even though the auditory stimuli provided less obvious cues during training than they did to other individuals. In a similar situation, a monkey with severely impaired somatosensory function was unable to learn the psychophysical task for assessment of vibration thresholds until a large vibratory stimulus applied to his upper arm was used as the detection stimulus during training (Rice and Gilbert, 1995).

Comparison of changes in auditory function between 11 and 19 years also provides evidence for an increase in impairment in methylmercury-exposed monkeys relative to controls. This is readily apparent for middle-dose monkey 118, whose right-ear function deteriorated from within control range to clearly above it during the 8-year period. The data available for low-dose monkey 104 also indicated normal pure-tone detection thresholds at 11 years, which was clearly not the case at 19 years. There was a suggestion in the three monkeys that were tested at the higher frequencies at both ages that higher frequencies were relatively more impaired at the older age compared with their younger data, although the absolute frequencies were not necessarily more elevated compared with controls. The hearing deficits observed in the current study presumably represent irreversible hearing loss, since they were present 7–15 years after cessation of methylmercury exposure. Since auditory function was not tested during the period of dosing, it is not known whether this loss appeared during the period of methylmercury exposure or after cessation of dosing for those individuals with thresholds already elevated when first tested. However, it is also clear that individuals at the lower doses displayed normal thresholds at some frequencies at
11 years that were elevated at 19 years. The data suggest that impairment in the high-dose monkeys, in addition to being more severe, also had an earlier onset than in the lower-dose individuals: high-dose monkey 101 presumably had severely compromised auditory function at 11 years of age, and high-dose monkey 102 also displayed greater impairment compared with controls at 11 years than did the three monkeys at the lower doses. The apparent delayed neurotoxicity in some individuals, and relative acceleration of neurotoxic impairment in others, is consistent with findings in Japan (Harada, 1995). Persons with early MD as a result of relatively high exposure exhibited a worsening of some signs years after exposure to methylmercury ceased, while other individuals with lower exposure developed signs of methylmercury poisoning years after cessation of exposure. It also extends previous findings in our laboratory in a cohort of methylmercury-exposed monkeys in which overt clumsiness was first observed at 13 years of age, 6 years after cessation of methylmercury exposure (Rice, 1989b).

Average mercury levels in whole blood of unexposed persons (i.e., with no known occupational exposure and with little fish consumption) are in the range 4–20 ppb in various studies (WHO, 1990), clearly much lower than blood mercury concentrations of the monkeys in the current study. However, maximum blood mercury concentrations in adults who consume significant amounts of fish were reported to be as high as 800 ppb in individuals in Japan and 650 ppb in Sweden (WHO, 1990). The steady-state blood mercury concentrations of all five monkeys in the current study were at or below the maximum observed in humans and therefore are representative of individuals from fish-eating populations. Mercury concentrations at birth were higher than steady-state levels for all monkeys, which is also observed in humans (Amin-Zaki et al., 1974, 1976). Even these acute peak levels were below the maximum in adults in the above studies for two of the monkeys. Brain:blood mercury ratios following chronic methylmercury exposure in monkeys range from 2.5 to 3.0 (Burbacher et al., 1990; Rice, 1989c). While the brain:blood ratio has been reported to be greater than this in humans (Burbacher et al., 1990), this may be an artifact of delayed sampling (death) following cessation of methylmercury exposure and the longer half-life of mercury in brain compared with blood (Rice, 1989c). In any event, mercury levels in the target organ (brain) in the monkeys in the current study during dosing were probably similar to those of highly exposed humans.

The site(s) of damage responsible for the deficits observed in the current study is unknown. While hearing impairment and even deafness have been observed as a consequence of methylmercury exposure in humans since the 1960s, pathological assessment of human or monkey tissue has not included the auditory system. While a few studies have been performed in other animal models (Ramprashad and Ronald, 1977; Anniko and Sarkady, 1978; Falk et al., 1974; Wassick and Yonovich, 1985), exposure was acute high dose, which is probably irrelevant to the current study or typical human exposure. The primary auditory cortex of the macaque lies in the depths of the sylvian fissure on the middle one-third of the superior temporal plane, while much of the rest of the superior temporal gyrus is also auditory. Removal of the primary and surrounding cortex in macaques results in deficits in pure-tone detection threshold (Heffner and Heffner, 1990). Consistent with the results of the current study, deficits were found to be greatest throughout the middle range of frequencies, with high (32 kHz) and low frequencies relatively spared. The pattern of hearing loss in the current study differs somewhat from the results of a previous study in our laboratory in which monkeys were exposed to 50 µg/kg/day mercury as methylmercury from birth to 7 years of age (Rice and Gilbert, 1992). In that study, high-frequency hearing was preferentially damaged when monkeys were tested at 14 years of age. The fact that impairment was observed over most frequencies in some individuals in the current study may reflect the diffuse pattern of damage observed following in utero exposure (Takeuchi and Eto, 1975; Takeuchi, 1968). In addition, however, there was evidence of relatively greater impairment of higher frequencies with increasing age in some individuals, suggesting that there may be more than one neurotoxic process underlying the observed deficits.

The monkeys in the current experiment also underwent assessment of visual function at 4–6 years of age and somatosensory function at 15 years of age. High-dose monkey 102, middle-dose monkey 116, and low-dose monkey 104 exhibited extreme impairment in spatial visual function, with the other two monkeys having normal spatial vision (Rice and Gilbert, 1990). All treated monkeys displayed elevated thresholds for detection of vibration at the fingertips, with no indication of a dose-related deficit (Rice and Gilbert, 1995; Rice, 1996). As in the current study, there was no indication of a cognitive deficit as assessed by the monkeys' ability to learn the task and produce orderly, stable psychophysical data.

**SUMMARY**

Monkeys exposed to methylmercury in utero to 4 years of age exhibited elevated detection thresholds for pure tones at 11 and/or 19 years of age compared with age-matched controls. Deficits were observed at most frequencies tested, including the range of frequencies used in human speech. While blood mercury levels during the period of exposure were at or near the upper boundary of persons ingesting large amounts of fish, a recent study in children in which deficits in auditory processing were observed suggests that subtle deficits in auditory function may result from lower-level exposure. There was evidence for delayed neurotoxicity in the auditory system being manifested between the ages of 11 and 19 years in low-dose individuals and acceleration of existing impairment in higher-dose individuals when compared with controls. These findings extend previous data from our laboratory in which delayed manifestation of overt toxicity was observed in another cohort of methylmercury-exposed monkeys, and are consistent with findings in persons with Minamata disease.
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REFERENCES


