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Neurophysiologic measures of auditory function in fish consumers: associations with long chain polyunsaturated fatty acids and methylmercury

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Abstract

Background—Determining if associations exist between child neurodevelopment and environmental exposures, especially low level or background ones, is challenging and dependent upon being able to measure specific and sensitive endpoints. Psychometric or behavioral measures of CNS function have traditionally been used in such studies, but do have some limitations. Auditory neurophysiologic measures examine different nervous system structures and mechanisms, have fewer limitations, can more easily be quantified, and might be helpful testing additions. To date, their use in human epidemiological studies has been limited. We reviewed the use of auditory brainstem responses (ABR) and otoacoustic emissions (OAE) in studies designed to determine the relationship of exposures to methyl mercury (MeHg) and nutrients from fish consumption with neurological development. We included studies of experimental animals and humans in an effort to better understand the possible benefits and risks of fish consumption.

Objectives—We reviewed the literature on the use of ABR and OAE to measure associations with environmental exposures that result from consuming a diet high in fish. We focused specifically on long chain polyunsaturated fatty acids (LCPUFA) and MeHg.

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Methods—We performed a comprehensive review of relevant studies using web-based search tools and appropriate search terms.

Results—Gestational exposure to both LCPUFA and MeHg has been reported to influence the developing auditory system. In experimental studies supplemental LCPUFA is reported to prolong ABR latencies and human studies also suggest an association. Experimental studies of acute and gestational MeHg exposure are reported to prolong ABR latencies and impair hair cell function. In humans, MeHg exposure is reported to prolong ABR latencies, but the impact on hair cell function is unknown.

Conclusion—The auditory system can provide objective measures and may be useful in studying exposures to nutrients and toxicants and whether they are associated with children's neurodevelopment.

Keywords

Methylmercury; Fish Consumption; LCPUFA; Auditory Brainstem Response

1. Introduction

Fish consumption is an issue of significant public health importance since fish provides the daily nutritional needs of over three billion people worldwide (FAO, 2008). Fish contain nutrients critical for neural development and are the primary human source for the long chain polyunsaturated fatty acids (LCPUFA) that are essential for brain development. However, fish also contain the toxicant methylmercury (MeHg) that in adequate quantities is known to adversely affect fetal and child development (Mozaffarian and Rimm, 2006, Strain et al., 2004). Presently the risks and benefits of fish consumption during pregnancy as related to child development are not well established (Budtz-Jorgensen et al., 2007, Davidson et al., 2008, Oken et al., 2005). Some observational epidemiological studies have indicated that levels of exposure to MeHg achieved by fish consumption may influence children's development while others have not supported that conclusion (for a review see Oken and Bellinger, 2008).

Most epidemiological studies of human exposure to toxicants and nutrients have used traditional developmental measures of CNS function. These include psychometric and behavioral tests that reflect performance on tasks involving cognition, perception, memory, motor functions, scholastic achievement and social behaviors. These are indirect measures of brain function known to be influenced by a variety of factors such as socioeconomic position, home environment, parental intelligence, etc., that investigators cannot control and which complicate the analysis of the findings. Auditory neurophysiology measures such as auditory brainstem responses and otoacoustic emissions are more direct measures of neural activity and appear to be independent of such influences (Lonsbury-Martin and Martin, 1990, Moore et al., 1995). In this review we focus on the association of exposures to nutrients present in fish, specifically LCPUFA, and MeHg as they relate to peripheral and brainstem auditory physiological measures. Nearly all studies carried out to date place their emphasis on these structures.

1.1 Fish Consumption and Nutrients

Fish are the primary source of nutrients such as LCPUFA, which play a critical role in neurodevelopment (Marszalek and Lodish, 2005). LCPUFA such as n-3 and n-6 fatty acids are required in the developing brain for membrane fluidity and structure, signaling, neurotransmitter production and retinal membrane development among others (Brenna and Lapillonne, 2009, Eilander et al., 2007, Yehuda, 2003). Docosahexaenoic acid (DHA; 22:6 n-3) and arachidonic acid (AA, 20:4 n-6) are two fatty acids that play a crucial role in this

development (for a review see Yehuda, 2003). DHA is found in abundance in marine animals, compared to the saturated and mono-unsaturated fats found in most land-dwelling animals (Crawford et al., 2001, Hibbeln et al., 2006, Simopoulos, 2006).

The developing brain has a significant requirement for essential LCPUFA both prenatally and during early postnatal life (Diau et al., 2005, Martinez, 1992). The majority of this demand occurs during the third trimester when the brain is growing rapidly, and continues through the first two years of life (Lapillonne and Jensen, 2009). Adequate maternal nutrition is required even though the placenta biomagnifies LCPUFA concentrations to enrich the fetal supply (Chambaz et al., 1985). However, the human body is unable to synthesize adequate quantities of these essential fatty acids and they must be obtained from dietary sources (Brenna and Lapillonne, 2009).

1.2 Fish Consumption and Methylmercury Exposure

All fish also contain small amounts of MeHg. Inorganic mercury present in the aquatic environment is methylated by bacteria and bio-accumulates up the food chain. Small reef fish typically contain less than 0.5 ppm MeHg, while large ocean fish can contain 1 ppm or more (WHO, 1990). Methylmercury can be neurotoxic to the developing brain in adequate doses, but the level of exposure where harmful effects begin to occur is presently not known (Clarkson, 1995, Davidson et al., 2006). Fetal neurologic effects seen in the overt poisonings that occurred in Japan and Iraq included severe developmental and intellectual disabilities (Bakir et al., 1973, Mizukoshi et al., 1975). The effects, if any, of lower-dose exposures are less clear. The only confirmed cases of prenatal MeHg poisoning from fish consumption followed industrial pollution in Japan in the 1950s and 1960s. The MeHg in fish at Minamata exceeded 35 ppm (Harada, 1995). This concentration is over 60 times higher than levels normally present in ocean fish, and has not been reported since the poisonings in Japan.

Two large-scale epidemiological studies examining the association between child development and MeHg exposure from fish consumption are taking place in the Faroe Islands and the Seychelles Islands (Debes et al., 2006, Myers et al., 2003). These two studies differ in exposure source and co-exposures. In the Faroe Islands MeHg exposure is primarily from whale consumption, but fish are also consumed and there is high concomitant exposure to polychlorinated biphenyls (PCB) (Grandjean et al., 2001). Faroes investigators have reported statistical associations of MeHg with “language, attention, memory, and to a lesser extent in visuospatial and motor functions” (Grandjean et al., 1997). In the Seychelles Islands dietary exposure is only from fish consumption, there is no PCB exposure, Hg levels are higher (maternal hair level 6.9 ppm vs 4.3 ppm in the Faroes), and no consistent pattern of adverse developmental associations has been found (Davidson, Myers, 2006, Myers et al., 2007).

1.3 Quantitative Auditory Neurophysiology

Advances in neurophysiologic methods have provided the tools to objectively measure even subtle effects of toxic exposures if they are present (Araki et al., 1997). The auditory system is especially suited for epidemiological studies since many of the tests can be carried out in the field, and it allows precise measurement of peripheral end-organ function, brainstem transit speed, and central nervous system (CNS) function. Numerous neurophysiologic and behavioral measures have been used to assess the function of the auditory system, but this review focuses on ABRs and OAEs (Jacobson and Jacobson, 2004). Auditory brainstem responses (ABRs), also called brainstem auditory evoked responses (BAERs), represent the summed electrical potentials of sequential activation in the auditory system in response to a repeated stimulus such as a tone burst or click. The stimulus is presented at a rapid rate (e.g.

10 to 70 Hz), and an EEG is recorded from scalp electrodes. Averaging of several hundred stimulus responses or more is required to acquire the ABR as it is a far-field potential with maximum amplitudes in the microvolt range using external-leads in humans and microvolt range using needle electrodes in experimental animals. The ABR waveform has peaks and troughs and represents the summed action potential propagating from the auditory nerve through the cochlear nucleus to the inferior colliculus (Moller et al., 1982). ABR outcome measures include the threshold (the minimal amplitude where the ABR is first detected), amplitude (related to the number of neurons firing), latency (time from supra-threshold stimulus presentation to the waves peak, a measure of transmission speed) and the interpeak latency (time between the peaks).

Abnormalities in measured ABRs are related to structural changes in the cochlea and brainstem. ABR latency corresponds to the degree of myelination in the auditory pathway (Ito et al., 2004, Knipper et al., 2000, Moore, Perazzo, 1995). It also reflects synapse maturation and axonal structure (Rance, 2005, Wang and Manis, 2005). Body temperature is known to influence latencies and investigators generally control for it in animal experiments. Animals with central myelin deficiency from birth demonstrate broader ABR peaks and significantly prolonged latencies compared to controls (Ito, Tokuriki, 2004, Naito et al., 1999). Animals with abnormal myelination and demyelination also show abnormal ABR latencies (El-Badry et al., 2007, Roncagliolo et al., 2000) and neurofilament deficiencies and altered axonal structure have been shown to result in ABR conduction deficiencies independent of myelin changes (Jones et al., 2008, Sheykhosslami et al., 2001). In humans, ABR maturation and latency prolongation has been associated with latent iron deficiency, possibly reflecting subtle changes in myelination (Amin et al., 2010).

Otoacoustic emissions (OAEs) measure sound pressure variations in the ear canal and are thought to reflect induced movement of the basilar membrane by outer hair cells (OHCs) (Kemp et al., 1990, Lonsbury-Martin and Martin, 1990). Measuring OAEs provides a quantitative assessment of the function of the peripheral auditory system, which cannot be obtained by measuring ABRs alone. OAEs can be evoked by clicks or tones, termed click-evoked (CE) or distortion product (DP) OAEs, and can also occur spontaneously. OAE assessment is commonly used to screen hearing in newborns and children because of its rapid administration and high sensitivity (Jacobson and Jacobson, 2004). OAEs are decreased or absent when OHCs are missing or non-functioning (Colombari et al., 2011, Liberman et al., 2002).

1.4 Auditory Toxicity

ABRs have been used to quantify damage to the developing auditory system caused by toxicants such as heavy metals and organic compounds (see Dyer, 1985, Otto et al., 1988). Children with elevated blood lead concentrations are reported to have elevated ABR thresholds and prolonged latencies (Rothenberg et al., 2000, Zou et al., 2003) at exposure levels below the EPA-recommended 10 µg/dL (Otto et al., 1985, Schwartz and Otto, 1991). Exposure to polychlorinated biphenyls (PCBs), halogenated hydrocarbons ubiquitous in the environment, is also associated with decreased ABR amplitudes (Herr et al., 1996) and elevated ABR thresholds (Powers et al., 2009). Human studies examining prenatal PCB exposure have reported abnormal ABRs (Chen and Hsu, 1994) and an association between exposure and behavioral pure-tone thresholds at several frequencies (Grandjean, Weihe, 2001). Exposure to ethanol *in utero* in animal experiments has been reported to elevate ABR thresholds and cause cochlear abnormalities resulting in congenital sensorineural hearing loss (Church et al., 1996).

OAEs can also provide a quantitative assessment of outer hair cell function in the setting of toxic exposure. Exposure to PCBs is reported to cause DP-OAE amplitude decreases at low

frequencies with preservation at high frequencies in rats (Lasky et al., 2002). Many commonly-used pharmaceuticals such as aminoglycoside and macrolide antibiotics, chemotherapeutic agents and non-steroidal anti-inflammatory agents can be ototoxic and cause OAE amplitude changes (Yorgason et al., 2006). Environmental noise exposure can disrupt auditory function causing both threshold shifts in ABRs and lower OAE amplitudes (Daniel, 2007, Marshall et al., 2009).

2. Methods

Relevant studies were identified using PubMed (National Center for Biotechnology Information, 2011), Google Scholar (Google, 2011) and Web of Science (Thomson Reuters, 2011). Search terms included combinations of the following: “auditory brainstem response,” “brainstem auditory evoked potential,” “otoacoustic emissions,” “auditory hair cells,” “methylmercury,” “Minamata Disease,” “unsaturated fatty acids,” “docosahexaenoic acids,” “dietary fats” and “fish consumption.” Studies include reports on animal models, human case studies, retrospective and prospective epidemiological studies as well as randomized controlled trials. Based on reports of auditory toxicity from other exposures, associations with LCPUFA and MeHg are hypothesized to be subtle if they are present. Therefore conclusions from case studies or studies with small cohorts are difficult to interpret but are included for completeness.

3. Results

3.1 Auditory Function and LCPUFA

3.1.1 Animal Studies—Supplementation with n-3 LCPUFA is reported to be associated with changes to the ABR in the developing auditory system. We found no animal studies that examined cochlear structure or measured otoacoustic emissions to assess hair cell function. All ABR studies were done in rats. Table 1 summarizes the impact of dietary DHA on neurodevelopment. With one exception these studies found *detrimental* effects of supplementing dietary LCPUFA.

Rat pups born to dams fed a diet deficient in alpha-linolenic acid (ALA), an 18-carbon n-3 precursor, had ABR amplitudes and latencies at 6 months of age similar to 18-month-old control rats suggesting premature aging (Bourre et al., 1999). Wave I-III and I-IV interpeak latencies were prolonged in pups born to dams fed excess DHA (Church et al., 2008, Church et al., 2009, Haubner et al., 2002, Saste et al., 1998, Stockard et al., 2000). Additionally the auditory startle reflex (ASR), a measure of the ability of a loud stimulus to elicit a startle response, was delayed in the supplemented group which matches the reported ABR conduction delays (Saste, Carver, 1998). ABR thresholds were elevated with DHA supplementation and these elevations persisted >16 months after supplementation ceased (Church et al., 2010, Church et al., 2007).

Postnatal formula supplementation with DHA + AA resulted in longer latencies compared to supplementation with DHA alone or to the control group (Auestad et al., 2003). However the application of experimental animal studies to human nutrition ones is limited by differences in auditory development and doses provided.

Many animal studies provide supplemented food *ad lib* to the animals making accurate measures of actual LCPUFA intake difficult. Maternal breast-milk LCPUFA content has been used to relate animal values to human data. In unsupplemented humans the highest levels are seen in predominantly fish-eating populations such as Japan (0.99 – 1.10 %) and the Canadian Arctic (1.40 %) (Brenna et al., 2007, Yuhas et al., 2006). As seen in Table 2, DHA levels in milk varied depending on the supplemented LCPUFA dose. Some levels

were in the range of unsupplemented humans (Haubner, Stockard, 2002), while others were substantially elevated and unlikely to be naturally-occurring. One study reported the weekly food consumption for each experimental group, allowing the calculation of an average daily intake of 212 mg of DHA in the ‘Excess DHA’ group (Church, Jen, 2007). In an average-weight lactating rat, assuming a total body surface area (TBSA) of 390 cm² using Meeh’s formula (Gouma et al., 2012, Hanson et al., 2001) the “Excess DHA” group’s dose corresponds to approximately 5.4 grams per m². For an adult human the equivalent dose is just over 11 grams of DHA daily (Verbraecken et al., 2006). For comparison, recent reports suggest an adequate human daily intake of DHA during pregnancy is 200 mg (Koletzko et al., 2008).

Although experimental studies provide a convenient, controlled model for studying the impact of LCPUFAs on auditory function, translating these results to humans is challenging because of variations in dosing, neural development and other physiologic differences between animals and humans (Hadders-Algra, 2008). For example, in the rat auditory brainstem myelination begins around the 7th postnatal day of life (Church et al., 1984, Rozeik and Von Keyserlingk, 1987), but in humans myelination begins around the 26th gestational week and is present in all auditory neural tracts by the 29th gestational week (Bayer et al., 1993, Moore, Perazzo, 1995, Roncagliolo et al., 1998). These timing differences may have significant implications in relation to prenatal versus postnatal exposure.

3.1.2 Human Studies—Term infants fed formula not supplemented with LCPUFA were found to have prolonged ABR wave I latencies compared to breast milk-fed infants (Khedr et al., 2004). No latency differences were reported in preterm infants randomized to supplemented versus unsupplemented formulas (Faldella et al., 1996). Two studies demonstrated that ABR maturation is accelerated in the presence of increased dietary LCPUFA (Amin et al., 2000, Unay et al., 2004). These studies examined the rate of latency decrease during the early postnatal period rather than at a single time-point. An accelerated decrease was observed in breast-fed versus unsupplemented formula-fed infants. In a prospective trial an accelerated decrease was observed in DHA-supplemented versus unsupplemented infants. These results suggest that increased early DHA intake modifies auditory processing, but the studies did not account for *in utero* effects.

We identified only one study that examined the associations of prenatal maternal LCPUFA on auditory evoked potentials in infants (Parra-Cabrera et al., 2008). Maternal AA and DHA intake was calculated from a diet questionnaire, which had previously shown good correlation between LCPUFA intake and erythrocyte fatty acid composition. The authors grouped LCPUFA values into “high” and “low” intake based on the study median. ABRs were recorded in the infants at a median age of 36 days. Subjects were grouped into “long” or “short” latency clusters based on wave and interpeak latencies. Maternal AA intake (and not DHA intake) was significantly elevated in the short latency group. They used a multivariate logistic regression model to adjust for cofactors and found the odds ratio for belonging in the short latency group to be 3.6 among infants born to mothers with an AA-rich diet suggesting improved neural transmission.

The mechanism by which LCPUFA might affect auditory function is unknown. In animal models n-3 LCPUFA supplementation resulted in decreased myelin basic protein and CNPase activity, a marker of myelinogenesis (DiBiase and Salvati, 1997, Salvati et al., 1996). These two markers suggest that a component of ABR latency changes is related to abnormal myelination (Moore, Perazzo, 1995). Moreover, maternal DHA supplementation was reported to alter myelin lipid composition in developing pups (Haubner et al., 2007). In addition, LCPUFA might be critical in modulating auditory function at the level of the hair

cells. An important physiologic action of AA is the inhibition of A-type potassium channels (Boland et al., 2009), a subset of which (Kv4.2) has been identified in the outer hair cells of the chick cochlea (Sokolowski et al., 2004). This channel is AA-sensitive, although the mechanism of AA modification and the potential effects of dietary AA on transduction are unclear. More recently, fatty acid binding proteins (FABPs) critical to the uptake of fatty acids (Coe and Bernlohr, 1998) have been found to be localized in the mammalian Organ of Corti (Saino-Saito et al., 2010). Other postulated mechanisms of action include increased oxidative stress, apoptosis, and epigenetic changes in the fetus that persist throughout life (Church, Jen, 2010). Regardless of the mechanism, these findings suggest that complete evaluation of the relationship between auditory function and LCPUFA intake should include examination of both ABR and OHC function.

3.2 Auditory Function and Methyl Mercury Exposure

Following the Minamata disaster in the 1950s, animal models were created to replicate acute, high-dose postnatal MeHg exposure. More recently auditory endpoints have been studied in models of MeHg exposure during gestation and lactation (see Table 3 for an overview of animal studies).

Postnatal MeHg exposure in adequate dosage can disrupt myelination and damage the adult central auditory pathways. Rats exposed to high-dose MeHg show Wallerian-like degeneration and eventual myelin breakdown in the peripheral nervous system (Herman et al., 1973). Similar results are seen in neurons and supporting cells of the temporal cortex in non-human primates (Zook and Wilpizeski, 1980). A review of fetal Minamata disease suggests that abnormal myelin profiles are present following *prenatal* MeHg exposure, and that this damage might affect the functional properties of neurons in the CNS (Reuhl and Chang, 1979).

3.2.1 Animal Studies of Prenatal MeHg Exposure—Auditory function studies following animal models of prenatal exposure to MeHg are equivocal. Three studies demonstrated impaired auditory startle reflex following gestational exposure, requiring more intense startle elicitors (suggesting higher thresholds) and reporting prolonged latencies (Beyrouthy and Chan, 2006, Buelke-Sam et al., 1985, Geyer et al., 1985). Two reports of longitudinal trials in non-human primates demonstrate elevated behavioral thresholds many years following exposure suggesting MeHg induces permanent changes in the developing brain (Rice, 1998, Rice and Gilbert, 1992). Hearing loss was reported across the full range of tested frequencies at ages 11 and 19 years in the monkeys that were exposed prenatally and through age 4 years. In a separate study, monkeys exposed chronically to low levels of MeHg (they had steady state blood Hg levels of 600 to 900 ppb) from birth through adulthood exhibited high-frequency hearing loss.

Two recent studies examined ABR thresholds and latencies following prenatal exposure. The first study exposed rats to either 1.5 or 3.0 ppm MeHg in drinking water and reported no changes in ABR threshold or peak latencies and also no changes in DP-OAE amplitudes, suggesting normal hair cell function (Powers, Poon, 2009). The second study orally gavaged mice with 20 µg/kg/day of MeHg and reported that ABR thresholds were elevated and peak latencies were prolonged. However, the affected waves differed depending on whether exposure continued through lactation or ceased at birth (Huang et al., 2011). Brain total mercury levels of the mice with continued exposure through lactation ranged from 100–150 ppb and were comparable to levels seen in Seychellois children whose mothers were presumed to consume a diet high in fish (50–200 ppb, see Lapham et al., 1995).

3.2.2 Animal Studies of Postnatal MeHg Exposure—Functional auditory impairments have also been described in animals exposed to MeHg postnatally. Cochlear microphonic (CM) and whole-nerve action potentials, direct measures of auditory function, demonstrate reduced amplitudes and increased latencies in cats (Shimoyama and Eto, 1973) and guinea pigs (Hotta et al., 1997, Konishi and Hamrick, 1979). Behavioral measures of auditory function using startle inhibition demonstrated impaired responses in adult exposed rats which improved following cessation of exposure (Wu et al., 1985). The authors argue that this improvement precludes hair cell loss as the cause because damaged hair cells do not recover in mammals. Acoustic startle reflex latencies increased and reactivity decreased in adult exposed rats (Vezér et al., 2005). In adult mice exposed to MeHg ABRs demonstrate adverse effects such as elevated thresholds and prolonged latencies, especially of wave V (Chuu et al., 2001, Huang et al., 2008a, Wassick and Yonovitz, 1985). In adult rats exposed to MeHg cortical auditory evoked potentials suggest prolonged latencies and decreased response durations (Vezér, Papp, 2005), results similar to those of animals exposed to inorganic mercury (Arezzo et al., 1985, Desi et al., 1996). These measures of auditory system function are provided for comparison, but no human studies of MeHg exposure were identified which utilized these electrophysiologic markers.

Postnatal MeHg exposure also can disrupt hair cell structure and cause cell damage. Hair cell loss has been reported across the entire cochlea following acute high-dose MeHg exposure in guinea pigs (Falk et al., 1973, Falk et al., 1974), rats (Sato et al., 1986) and harp seals (Ramprasad and Ronald, 1977). In addition, the hair cells remaining show evidence of stereocilia disarray, lacking the characteristic V-formation, suggesting potential loss of function prior to hair-cell loss. These results contrast with high-frequency hearing loss identified in adult human cases of Minamata disease in Japan, which the authors suggest is because of species differences (Falk, Klein, 1974). We did not identify any human or animal studies examining hair cell function using OAEs.

3.2.3 Human Studies of Prenatal MeHg Exposure—Subjects with prenatal MeHg poisoning were examined following exposure to MeHg-tainted seed grain in Iraq and some revealed auditory deficits. One study of mother-infant pairs reported that about one in ten mothers demonstrated hearing impairment, while one of four was found to have severe hearing loss by clinical exam during infancy (Amin-Zaki et al., 1974). A reported prenatal MeHg poisoning in the US from maternal consumption of contaminated pig meat also resulted in impaired hearing (Brenner and Snyder, 1980). These reports are consistent with the animal data that suggests elevations in hearing thresholds occur in at least some prenatal exposed individuals.

Several cohort studies have examined associations of prenatal MeHg exposure from seafood consumption with auditory measures. Results from these studies are summarized in Table 4. A longitudinal cohort was established in the Faroe Islands to track children born to mothers consuming pilot whale meat and blubber as well as ocean fish. Cord blood and maternal hair mercury levels were measured at delivery, and the child's hair mercury was measured at each testing age. Children were recalled at ages 7 and 14 years for a battery of tests that included the ABR. At the seven-year evaluation, the authors reported an association between wave III and V latencies and cord mercury levels. Increased cord mercury was associated with prolonged latencies (Grandjean, Weihe, 1997). In a secondary analysis on a subcohort at seven years, wave III latency and I-III interpeak latency were reported to be significantly associated with cord blood mercury (Murata et al., 1999a). The cohort was also exposed to PCBs, so the authors carried out separate analyses with PCBs alone and with PCBs and MeHg. When MeHg was not included in the analysis, waves III (at 40 Hz presentation rate) and V (at 20 Hz presentation rate) latencies were associated with PCB levels. When adjustment for MeHg was included in the analysis, only wave V latency was significant

(Grandjean, Weihe, 2001). At the 14-year test age, waves III and V latencies at both presentation rates were reported to be significantly associated with cord blood mercury. Additionally the child's current hair MeHg content was reported to be positively associated with wave III-V interpeak latency suggesting an association with recent postnatal MeHg exposure (Murata et al., 2004b). Because of the cohorts' prenatal PCB exposure the authors included PCB levels in the analyses and reported they "...did not affect any of the MeHg regression coefficients." These MeHg results are similar to the findings recently reported in a mouse gestational model, where wave III and V latencies were prolonged with prenatal MeHg exposure (Huang, Liu, 2011).

Several other cross-sectional studies have reported various associations between mercury exposure and ABR latencies. In Madeira ABRs were recorded in seven-year-old children and their association with recent maternal hair mercury levels examined (Murata et al., 1999b). This study (N=113) reported that the maternal hair levels significantly predicted wave III and V latencies as well as wave I-V and I-III interpeak latencies. A similar study in seven-year-old children from a Japanese fishing village examined the association of ABR latencies with recent maternal hair levels (Murata et al., 2004a). In this cohort (N=210) the authors found no significant associations between ABR wave latencies and the recent maternal hair mercury level.

A study in Greenland examined ABRs at 20 and 40 Hz in 40 children when they were between the ages of 6 and 12 years. No statistically significant associations were reported with MeHg exposure measured in cord blood or recent child or maternal hair values (Weihe et al., 2002). The same authors performed the studies in the Faroe Islands and Madeira and were able to compare the association of ABR latencies among the three study locations with maternal hair mercury levels. They reported "Mercury was found to be a significant predictor for the latencies of the BAEP [an alternate term for the ABR] I-III and I-V intervals..."

The results from these studies suggest an association between prenatal MeHg exposure and auditory function. However, the use of recent maternal hair values measured years following birth as a biomarker for prenatal exposure complicate that interpretation and suggest that a study with prenatal measurement of exposure is indicated. All of these studies focused on contaminant exposure and did not consider possible associations with nutrients present in fish on auditory function. Maternal hair mercury is reported to correlate well with maternal DHA status in a population of fish consumers and should be considered in future studies (Davidson, Strain, 2008).

3.2.4 Human Studies of Postnatal MeHg Exposure—Hearing loss is a major characteristic of Minamata disease (Fujisaki et al., 1971, Ino and Mizukoshi, 1977, Kurland et al., 1960, McAlpine and Araki, 1958, Nosaka et al., 1970). Audiometry and neuropathologic observations suggest a combination of cochlear (hair cell) and retro-cochlear dysfunction (Mizukoshi, Nagaba, 1975). Longitudinal follow-up of these patients has identified subgroups with either chronic hearing loss or with improved hearing which suggests that hair cell loss cannot fully explain the deficits observed (Harada, 1995, Mizukoshi et al., 1989, Uchino et al., 1995). A study in communities near Minamata Bay Exposure where exposure was to lower doses of MeHg reported an increased frequency of hearing loss in the population (Ninomiya et al., 1995). Prolonged ABR latencies have been recorded in patients diagnosed with Minamata disease, although exposure verification in these patients is not possible (Hamada et al., 1982, Inayoshi et al., 1993). The reported clinical findings are in agreement with animal studies of postnatal exposure which demonstrate equivocal cochlear versus retro-cochlear damage, elevated thresholds and prolonged latencies. Reports following accidental MeHg poisoning of Iraqi adults match the

reported hearing deficits from Japan. In Iraq, exposed adults demonstrated hearing loss more frequently with increasing exposure levels (Bakir, Damluji, 1973). However, on follow-up two years after the initial assessment some patients had regained their ability to hear (Al-Damluji, 1976).

Two cohorts of children in the Andean gold mining region of Ecuador have been reported to have ABR changes associated with their MeHg burden. Mercury levels are elevated in this region owing to the use of metallic mercury in gold mining and elevated MeHg levels in food. No prenatal mercury measurements were made, but recent child mercury levels were measured in blood. These studies measured ABRs in exposed children and correlated peak latencies with the children's blood mercury levels (see Table 4). In one study with 19 children and adults, the authors reported only "... borderline statistical significance for the I–III interpeak transmission time ($r = 0.454$, $p = 0.051$) on the left side" (Counter et al., 1998). In the other study there were 31 children and the author reported that wave V latency ($r = 0.38$, $p = 0.03$) and I–V interpeak latency ($r = 0.41$, $p = 0.02$) were significantly correlated with blood Hg (Counter, 2003). Additionally, when children were grouped into high and low blood Hg levels based on the median B-Hg value, wave V latency and wave III–V interpeak latency were reported to be prolonged in the "high" blood level group. A large cross-sectional study in US adolescents recently reported no associations between blood mercury levels and hearing loss (Shargorodsky et al., 2011), although the blood Hg levels in this study population are far below those reported following MeHg poisonings (WHO, 1990).

We found no studies that had measured OAEs in subjects with MeHg exposure. However, there is one case report of an adult poisoned by dimethylmercury (a highly toxic compound). She had a battery of auditory tests including pure-tone thresholds, OAEs and ABRs (Musiek and Hanlon, 1999). Her OAEs were normal except for decreased high-frequency amplitudes (2,781 Hz on the left and 4,375 Hz bilaterally). Audiometric testing revealed a mild high-frequency bilateral sensorineural hearing loss and ABRs showed prolonged wave I–V interpeak latencies bilaterally along with overall poor wave morphology. These results suggest that dimethylmercury exposure can result in damage to outer hair cells, especially those tuned to high frequencies.

4. Conclusions

Auditory neurophysiologic testing (ABRs and OAEs) can provide objective evidence of associations between toxicant and nutrient exposures when associations with exposures are present. High dose prenatal and postnatal MeHg exposure in experimental animals and humans is clearly associated with deficits in auditory function. However, an association between lower dose MeHg exposures such as those seen with fish consumption and objective electrophysiologic and acoustic measures is less definitive. Experimental studies with low dose exposure are inconsistent and their relation to human studies is unclear. The association of LCPUFA with auditory function has not been well studied in humans, but experimental studies suggest that excessive doses may have a detrimental association. These findings suggest that further studies examining the association of MeHg and PUFA exposure with neurophysiologic measures of the auditory system will be needed to clarify any relationship that may be present.

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Abbreviations used

AA	Arachadonic Acid
ABR	Auditory Brainstem Response
BAER	Brainstem Auditory Evoked Response
DHA	Docosahexanoic Acid
LCPUFA	Long-chain Polyunsaturated Fatty Acids
MeHg	Methylmercury
OAE	Otoacoustic Emissions

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Highlights

Auditory neurophysiologic testing can provide objective evidence of associations between toxicant and nutrient exposure and neurodevelopment.

Gestational exposure to both LCPUFA and MeHg can influence the developing auditory system.

Human MeHg poisoning can potentially cause auditory function deficits.

The associations of MeHg exposure at lower doses appear to be minimal if present.

There is a need for well-controlled, double blind prospective longitudinal studies of the relationship of MeHg and PUFA exposure on the auditory system.

Table 1

The effects of dietary LCPUFA consumption on the auditory system of animal models.

Author/Date	Animal Model	Diet Composition ^a	Diet Duration	Outcomes	Results
Saste et al., 1998	Rats, Sprague-Dawley	Reference diet, corn oil or fish oil (8.8% DHA) supplemented diet	Dams begun gestational day 1, fed prenatal & postnatal	Neurodevelopmental reflex appearance, ABR latencies (P23 & P29)	<ul style="list-style-type: none"> ASR delayed in fish oil (DHA) group ABR I-III latency prolonged in fish oil (DHA) group
Bourre et al., 1999	Rats, Wistar	Rodent diet with 6% lipids, either: ALA deficient (1.2 g LA/100g diet) or control (1.2 g LA + 0.2 g ALA/100g diet)	Diet begun 15 days prior to breeding. Pups fed same diet	ABR audiograms, latencies at 21 days, 6 mo ('adult'), 18 mo ('aged')	<ul style="list-style-type: none"> 18-mo control diet had high freq. hearing loss ALA-deficient adult rats had ABR amplitudes similar to aged control rats; latency changes NS but trend similar to aged controls
Stockard et al., 2000	Rats, Sprague-Dawley	Rodent diet supplemented with 2%, 4% or 6% DHA	Dams begun gestational day 2	Milk FA analysis, cerebrium FA analysis, ABRs (P24 & P31).	<ul style="list-style-type: none"> ABR I-IV latency prolonged in a dose-dependent manner with dietary DHA
Haubner et al., 2002	Rats, Sprague-Dawley	Rodent diet supplemented with 0%, 1% or 3% DHA	Dams begun gestational day 1, fed prenatally, postnatally; pups fed same diet at wean	Brainstem FA analysis, ABR latencies (P24 & P31), ASR appearance, brainstem CNPase activity	<ul style="list-style-type: none"> ASR delayed in 3% DHA group vs 0% group CNPase activity NS among groups ABR I-IV latency prolonged in 3% DHA group vs 0% group at both time points Brainstem AA levels inversely related to DHA
Auestad et al., 2003	Rats, Sprague-Dawley	Pups fed milk formulas with high DHA (2.3%), DHA + ARA (0.6% DHA, 0.4% AA) or essential LA/ALA only	Dams fed regular chow prenatally. At birth, pups fed one of three formulas. Control group suckled.	Weight at P28, ABR latencies at P28, Brainstem biochemical analysis	<ul style="list-style-type: none"> ABR I-IV latency shorter for High DHA vs DHA + AA groups, NS from LA/ALA formula. High DHA group had decreased norepinephrine in inferior colliculus High DHA group had elevated DHA, decreased AA in brainstem
Church et al., 2007	Rats, Wistar	Control (n-3/n-6: 0.14: 0% DHA, 7.8% ALA), Excess (n-3/n-6: 14.0: 9.1% DHA, 1.6% ALA, 0.9% AA), Deficient (n-3/n-6: ~0.0)	Dams begun gestational day 1, fed prenatally and postnatally	Weights (P0 & P24), ABR thresholds (P24)	<ul style="list-style-type: none"> No weight difference at birth; by P24 Excess < Deficient < Control ABR thresholds were as follows: Excess > Deficient > Controls ABR wave morphology abnormal in Excess group

Author/Date	Animal Model	Diet Composition ^a	Diet Duration	Outcomes	Results
Church et al., 2008	Rats, Wistar	(Same as above)	(Same as above)	Weights (P0 & P24), ABR latencies (P24)	<ul style="list-style-type: none"> ABR I-IV latency in Deficient, Excess groups were prolonged compared to controls
Church et al., 2009	Rats, Wistar	(Same as above)	(Same as above) At P24, all rats to rodent diet	Weights (~P170), ABRs (~P170)	<ul style="list-style-type: none"> No weight differences at test date No ABR latency effects; ABR thresholds increased in Excess group vs others; Excess group had increased ABR suprathreshold amplitudes
Church et al., 2010	Rats, Wistar	(Same as above)	(Same as above) At P24, all rats to rodent diet	Weight, Kaplan-Meier survival, ABRs (16 months)	<ul style="list-style-type: none"> Excess group had decreased weight & diminished survival ABR thresholds increased in Excess group ABR L-I profile shows 33% SNHL in Excess group

AA: Arachidonic acid (20:4 n-6); **ABR**: Auditory brainstem response (also BAER); **ALA**: -linolenic acid (18:3 n-3); **ASR**: Auditory startle reflex; **DHA**: Docosahexanoic acid (22:6 n-3); **FA**: Fatty acid; **IC**: Inferior colliculus; **L-I**: Latency-Intensity profile; **LA**: Linoleic acid (18:2 n-6); **NS**: Not significant; **P #**: Postnatal day #; **SNHL**: Sensorineural hearing loss

^aDiet compositions describe PUFAs as either g/100 g diet or %, which is % of FAs in diet (not % of total diet).

Table 2

Fatty acid content in breast milk reported from animal studies.

Reference	DHA in diet (% FA)	AA in diet (% FA)	DHA in Breast Milk (% FA)	FA Source
Brenna et al., 2007 (<i>human breast milk reference values</i>)	n/a	n/a	0.32 ± 0.22 % (mean ± SD) 1.40 % (maximum value)	Human breast milk (mean ± SD) Canadian arctic (maximum value)
Saste et al., 1998	0.0 % 8.8 %	0.0 % 1.1 %	0.3 ± 0.0 % 4.1 ± 0.4 %	Corn oil Fish oil
Bourre et al., 1999	n/a ^a	n/a ^a	Not Reported ^a	ALA-deficient; peanut oil ALA+LA: peanut oil & rape seed oil
Stockard et al., 2000	2.0 % 4.0 % 6.1 %	0.0 % 0.0 % 0.0 %	0.82 ± 0.19 % 1.43 ± 0.22 % 2.03 ± 0.19 %	Soy : Olive Oil blend (50:50 v/v) with DHA supplement
Haubner et al., 2002	0.0 % 0.8 % 3.2 %	0.0 % 0.0 % 0.0 %	0.45 ± 0.13 % 0.73 ± 0.12 % 1.83 ± 0.33 %	Soy : Olive Oil blend (50:50 v/v) with DHA supplement
Auestad et al., 2003	0.0 % 0.6 % 2.3 %	0.0 % 0.4 % 0.0 %	n/a ^b	Mixture of high-oleic safflower oil, fractionated coconut oil, soy & coconut oil with/without supplements
Church et al., 2007, 2008, 2009 & 2010	0.0 % 0.0 % 9.1 %	0.0 % 0.0 % 0.9 %	0.16 ± 0.04 % 0.23 ± 0.19 % 3.96 ± 0.23 %	Safflower oil (70 g/kg diet) Soybean oil (70 g/kg diet) Menhaden oil (70 g/kg diet)

AA: Arachidonic acid (20:4 n-6); ALA: -linolenic acid (18:3 n-3); DHA: Docosahexanoic acid (22:6 n-3); FA: Fatty acid; LA: Linoleic acid (18:2 n-6)

^aStudy compared ALA-deficient diet with ALA-LA diet; therefore DHA/AA in diet and breast milk were not measured.

^bFormula was provided directly to pups so no breast milk could be measured.

Table 3
Summary of animal auditory structure and function studies in the setting of mercury exposure.

Reference	Organic/Inorganic Hg	Exposure Timing	Animal Model	Endpoints	Findings
Nordberg & Serenius, 1969	Inorganic	Adult	Guinea Pig	Brain histology	Slow brainstem elimination
Anniko & Sarkady, 1978	Inorganic	Adult	Guinea Pig	Hair cells	Low-frequency hair cell loss
Desti et al., 1996	Inorganic	Adult	Rat	ECoG	Dose-dependent ECoG amplitude decrease with HgCl
Fazakas et al., 2005	Inorganic	Adult	Rat	Cortical EP	"Less pronounced" changes than Visual EP; data not shown
Huang et al., 2008	Inorganic	Adult	Mouse	ABR	Cinnabar (HgS) mirrors MeHg ABR latency shifts
Liang et al., 2003	Inorganic	Ex vivo	Guinea Pig	HC patch clamp	Potassium channels affected by HgCl
Herr et al., 2004	Inorganic	Gestational	Rat	ABR	No amplitude or latency effects
Falk et al., 1973	Organic	Adult	Guinea Pig	Hair cells	Frequency-variable hair cell loss, mainly low frequencies
Herman et al., 1973	Organic	Adult	Rat	Myelin histology	Wallerian-like degeneration, spiral ganglion necrosis
Shimoyama & Eto, 1973	Organic	Adult	Cat	CM, AP	Reduced amplitudes, increased latencies
Falk et al., 1974	Organic	Adult	Guinea Pig	Hair cells	Low-frequency hair cell loss
Ramprasad & Ronald, 1977	Organic	Adult	Harp Seal	Hair cells	Variable-frequency hair cell loss
Konishi & Hamrick, 1979	Organic	Adult	Guinea Pig	CM, AP, EP	CM suppression high-freq. AP tone suppression, EP normal
Zook & Wilpizeski, 1980	Organic	Adult	NHP	ABR, Cochlear histology	Threshold increased at 125 Hz, no temporal bone changes
Wassick & Yonovitz, 1985	Organic	Adult	Mouse	ABR	Thresholds increased, latencies trended to shorter
Wu et al., 1985	Organic	Adult	Rat	Startle Inhibition	Decrements in startle inhibition with exposure
Sato et al., 1986	Organic	Adult	Rat	Hair cells	OHC degradation
Igarashi et al., 1992	Organic	Adult	Rat	Cochlear histology	Minimal MeHg identified in cochlear tissue
Hotta et al., 1997	Organic	Adult	Guinea Pig	CM, AP, EP	CM output voltage differed, AP max output lower, EP diff
Chuu et al., 2001	Organic	Adult	Mouse	ABR	Threshold elevations, prolonged wave V latencies
Vezer et al., 2005	Organic	Adult	Rat	Acoustic Startle Reflex, PPI, ECoG	Exposure decreased ASR reactivity, increased latency; seemed to facilitate PPI; increased ECoG latency
Huang et al., 2008	Organic	Adult	Mouse	ABR	Wave V, I-V, III-V prolonged, thresholds elevated
Reuhl & Chang, 1979	Organic	Gestational	[Review]	[Review]	Disrupted myelin sheaths, degenerating axons
Buelke-Sam et al., 1985	Organic	Gestational	Rat	Acoustic Startle Reflex	ASR amplitude increased
Geyer et al., 1985	Organic	Gestational	Rat	Acoustic Startle Reflex	ASR impaired in exposed group
Rice, 1998	Organic	Gestational	NHP	Behavioral Thresholds	Elevated thresholds in all exposed, no freq specificity

Reference	Organic/Inorganic Hg	Exposure Timing	Animal Model	Endpoints	Findings
Beyrouty & Chan, 2006	Organic	Gestational	Rat	Acoustic Startle Reflex	Time to reach startle was greater in exposed pups
Powers et al., 2009	Organic	Gestational	Rat	ABR, DP-OAE	No MeHg DP-OAE or ABR latency or threshold effects
Huang et al., 2011	Organic	Gestational – Lactation	Mouse	ABR	Wave III, I–III prolonged in gestational, Wave V, III–V, I–V prolonged in gestational & lactation-only groups
Rice & Gilbert, 1992	Organic	Lactation – Adult	NHP	Behavioral Thresholds	High-frequency thresholds increased

ABR: Auditory brainstem response; **AP:** Whole-nerve (compound) action potentials; **ASR:** Auditory startle reflex; **CM:** Cochlear microphonics; **DP-OAE:** Distortion product otoacoustic emission; **EP:** Endocochlear potential; **ECOG:** Electrocochlogram; **HC:** Hair cell; **Hg:** Mercury; **HgCl:** Mercury Chloride; **HgS:** Mercury Sulfide; **MeHg:** Methylmercury; **NHP:** Non-human primate; **OHC:** Outer hair cells; **PPI:** Pre-pulse inhibition

Table 4
Human epidemiological studies examining association of ABR latencies to methylmercury exposure.

Author/Date	Study Location	Study Design	Subject Age	Sample Size	Exposure Measure	Exposure Results	Results
Murata et al., 2004	Japanese fishing villages	Cross-sectional	6.9 ± 0.3 (Mean ± SD)	210	Current maternal hair MeHg	Median maternal MeHg: 1.63 ppm	None significant
Grandjean et al., 1997	Faroe Islands	Longitudinal	6.83 (Median) ^a	824	Cord blood & maternal hair at parturition	Cord blood: 22.9 µg/L, hair: 4.27 ppm ^a	Wave III & V at both rates ^b
Murata et al., 1999	Faroe Islands	Longitudinal	n/a ^c	388	Cord blood & maternal hair at parturition	Cord blood: 23.0 µg/L, hair: 4.49 ppm	Waves III & I-III at both rates
Murata et al., 2004	Faroe Islands	Longitudinal	13.83 ± 0.32 (Mean ± SD)	859	Cord blood, maternal hair at parturition and current child hair MeHg	Cord blood: 22.6 µg/L, maternal hair: 4.22 ppm, child hair: 0.96 ppm	Waves III & V at both rates (cord blood); III & I-III at low rate (maternal hair); III-V at high rate (child hair)
Murata et al., 1999	Madeira fishing village	Cross-sectional	6.9 (Mean)	149	Current maternal hair MeHg	Geometric mean maternal hair MeHg: 9.64 ppm	Waves III, V, I-III and I-V (current maternal hair)
Weihe et al., 2002	Inuit community in Greenland	Cross-sectional	8.4 (Median)	31	Current maternal hair MeHg	Geometric mean maternal hair MeHg: 15.5 ppm	Results only reported in joint analysis with other studies
Counter et al., 1998	Ecuadorian gold-mining region	Cross-sectional	9.1 ± 2.9 (Mean ± SD)	10	Current child blood MeHg	16.2 ± 8.5 µg/L (mean ± SD) ^a	None significant
Counter et al., 2003	Ecuadorian gold-mining region	Cross-sectional	10.0 (Mean)	31	Current child blood MeHg	23.0 ± 18.9 µg/L (mean ± SD) ^a	Waves V & I-V (regression model), also I-III in high vs low MeHg groups

ABR: Auditory brainstem response; **MeHg:** Methylmercury

^aValues reported were for the entire study cohort, of which only a subset may have exposure or ABR measurements included in the reported analysis.

^bIn this study p-values < 0.10 were considered significant

^cSubset of subjects tested at 7 years of age, though no exact subject ages are reported