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Evidence of parallels between mercury intoxication and the brain pathology in autism

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The purpose of this review is to examine the parallels between the effects mercury intoxication on the brain and the brain pathology found in autism spectrum disorder (ASD). This review finds evidence of many parallels between the two, including: (1) microtubule degeneration, specifically large, long-range axon degeneration with subsequent abortive axonal sprouting (short, thin axons); (2) dentritic overgrowth; (3) neuroinflammation; (4) microglial/astrocytic activation; (5) brain immune response activation; (6) elevated glial fibrillary acidic protein; (7) oxidative stress and lipid peroxidation; (8) decreased reduced glutathione levels and elevated oxidized glutathione; (9) mitochondrial dysfunction; (10) disruption in calcium homeostasis and signaling; (11) inhibition of glutamic acid decarboxylase (GAD) activity; (12) disruption of GABAergic and glutamatergic homeostasis; (13) inhibition of IGF-1 and methionine synthase activity; (14) impairment in methylation; (15) vascular endothelial cell dysfunction and pathological changes of the blood vessels; (16) decreased cerebral/cerebellar blood flow; (17) increased amyloid precursor protein; (18) loss of granule and Purkinje neurons in the cerebellum; (19) increased pro-inflammatory cytokine levels in the brain (TNF-α, IFN-γ, IL-1β, IL-8); and (20) aberrant nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). This review also discusses the ability of mercury to potentiate and work synergistically with other toxins and pathogens in a way that may contribute to the brain pathology in ASD. The evidence suggests that mercury may be either causal or contributory in the brain pathology in ASD, possibly working synergistically with other toxic compounds or pathogens to produce the brain pathology observed in those diagnosed with an ASD.

Key words: autism, autism spectrum disorder (ASD), mercury (Hg), toxicity, brain pathology

INTRODUCTION

Evidence suggests that children with autism spectrum disorder (ASD) have a greater susceptibility to heavy-metal intoxication than typically developing children (Holmes et al. 2003, Kern and Jones 2006, Rose et al. 2008, Nataf et al. 2008, James et al. 2009, Geier et al. 2009a, Majewska et al. 2010, Youn et al. 2010, Kern et al. 2011a). For example, children with ASD have been found to have low plasma glutathione (GSH) and sulfate (SO_4) levels (Waring and Klovrza 2000, James et al. 2004, 2006, 2009, Geier and Geier 2006, Geier et al. 2009c, Pasca et al. 2009, Adams et al. 2011), both of which are critically important for detoxification (Gutman 2002, Kern et al. 2004). Expressions such as "poor detoxifiers" and "poor excretors" have been used in reference to those with ASD (Holmes et al. 2003). In a recent analysis, DeSoto and Hitlan (2010) found that there are 58 research articles which provide empirical evidence relevant to the question of a link between autism and one or more heavy metals. Of those 58 articles, 43 supported a statistically significant link between autism and exposure to toxic metals while 15 showed no statistically significant evidence of a link between metals and autism. Thus, 74% of the studies examined showed a significant relationship between ASD and toxic metals. Moreover, several recent studies have shown that the greater the toxic metal body burden in a child, the worse the autism symptoms that the child experiences

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(Holmes et al. 2003, Nataf et al. 2006, Geier and Geier 2007, Geier et al. 2009b, Adams et al. 2009, Kern et al. 2010, Elsheshtawy et al. 2011, Lakshmi Priya and Geetha 2011). Although studies have shown an association between autism and various toxic metals, such as cadmium, lead, and arsenic, the bulk of the research focused on mercury (Hg).

Mercury has a plethora of negative effects on the brain that are comprehensive and wide-ranging. In mercury intoxication, multiple systems are targeted. There does not appear to be one single target effect, but numerous consequences and cascades of events in the brain following mercury exposure. If mercury plays a causal or contributory role in the brain pathology of ASD, then the brain pathology seen in mercury intoxication should be similar to the brain pathology in ASD. Limited work has been done to examine or compile the similarities between the effects of mercury intoxication in brain and the pathology found in the brains of those with ASD. In 2000, Bernard and coauthors documented the similarities between the symptoms in autism and mercury exposure, and described brain changes relevant to both mercury and autism. However, since that time, an ever-increasing body of evidence elucidating the specific neurological effects of mercury on the brain has come to light.

This review of the recent literature reveals important parallels between the effects of Hg intoxication on the brain and the brain pathology found in ASD. Each section will explain the type of pathology evident from mercury intoxication and then the brain pathology associated with ASD. Each section will end with a summary statement. The discussion begins with mercury-induced morphological changes to the neuron.

MICROTUBULE AND NEURITE DEGENERATION

Evidence of microtubule and neurite degeneration from mercury exposure

Mercury can cause neuronal axons to degenerate because mercury disrupts the structure of the axon or neurite, causing it to break apart and depolymerize (Choi et al. 1981, Vogel et al. 1985, Leong et al. 2001, Castoldi et al. 2003). A critical structural component of the neurite membrane is tubulin, a globular protein (Leong et al. 2001). Under normal conditions, tubulin molecules link together (end to end) to form microtubules which provide the structure or scaffolding required by axons and dendrites (Yu et al. 2000, Leong et al. 2001). Guanosine triphosphate (GTP) continually binds to the tubulin and provides the energy that allows the tubulin proteins to remain linked together. However, when mercury is present in the brain, mercury binds to the GTP binding site of the beta subunit of the linked tubulin proteins, displacing the GTP. Because bound GTP provides the energy that allows the tubulin proteins to link and to remain linked together, the presence of mercury at the GTP binding sites stops the supporting energy transfer, which breaks the links between the tubulin subunits and disrupts this scaffolding. As a consequence, the microtubules break apart and the axons and neurites collapse or degenerate. This degeneration is also referred to as process retraction (Choi et al. 1981, Leong et al. 2001, Castoldi et al. 2003). The progressive degeneration is presumably mediated through mercury binding to free sulfhydryl groups both on the ends and on the surface of the microtubules (Castoldi et al. 2003). Mercury has a strong general affinity for, and binds with, sulfhydryl (-SH) groups. Moreover, mercury also has a high affinity for the sulfhydryls in the cytoskeletal proteins in neurons (Castoldi et al. 2003, Stoiber et al. 2004, Aschner et al. 2010). Furthermore, the effect that mercury has on microtubules and the subsequent axonal degeneration is unique to mercury. Other toxic metals, e.g. lead, manganese, cadmium, aluminum, do not show this effect (Leong et al. 2001).

This mercury-induced degeneration of the neurite caused by the binding of mercury to the tubulin has been shown in both *in vitro* and *in vivo* studies by several researchers. Leong and colleagues (2001), for example, applied a metal chloride solution (2 µl) of Hg (10^{-7} M) directly onto individual growth cones and found disruption of their membrane structure and linear growth rates in 77% of all nerve growth cones, disintegrated tubulin/microtubule structure, and neuronal somata sprouting failure. In other words, the axon degenerated. Vogel and coauthors (1985) documented that depolymerization (axon degeneration) occurred at concentrations above 1.0×10^{-5} M methylmercury (MeHg). MeHg was bound to free sulfhydryl groups exposed on the surface and at the ends of microtubules. Pendergrass and colleagues (1997) exposed rats to Hg^0 at concentrations present in the 'mouth air' of some humans with many amalgam fillings and found that by day 14 of exposure, the pres-

ence of tubulin was decreased by 41–74%. Pamphlett and Png (1998) looked for signs of damage to the motor and sensory neurons of mice that had been exposed to inorganic mercury and found that mercury "shrinks motor axons." The authors found that, after thirty weeks of exposure to either 1 or 2 μg/g of mercuric chloride, fewer large myelinated axons were seen in the Hg-injected groups than in the controls. They also found a slight increase in numbers of small axons in the posterior roots of mice exposed to 1 μg/g of Hg.

Importantly, the loss of large myelinated axons or the selective vulnerability of large axons reported in the Pamphlett and Png (1998) study has been shown by others. Stankovic and coauthors (2005), for example, examined the effects of Hg on motor neurons and found axonal degeneration, atrophy, and hypertrophy of axons, with large caliber axons being selectively vulnerable to the Hg. Another example is from a study by Stankovic (2006), who found atrophy principally to large myelinated fibers, a subpopulation of axons. Again, Mitchell and Gallagher (1980) had previously found methyl mercuric acetate (MeHgOAc) caused axonal degeneration in large myelinated fibers. It is important to note that projection or long-range neurons have, in general, bigger cell bodies and axons than local circuit (LC) neurons (Jacquin et al. 1989, Taylor 1996).

Neurites (axons and dendrites) provide the connections between neurons, and the connections between neurons form the neural circuitry of the brain. The connectivity of the neural circuitry allows for interaction within a brain region and between distinct brain regions. Retraction of processes or loss of these connective axons, as described above, leads to loss of connectivity in the brain. The following section examines the evidence for process retraction and abnormal connectivity found in autism.

Evidence of neurite degeneration/process retraction (loss of axons) and loss of connectivity in autism

Recent studies have shown evidence of process retraction or loss of axons in autism. Morgan and colleagues (2010), for example, examined the dorsolateral prefrontal cortex of male cases with autism (n=13) and control cases (n=9), and found process retraction and thickening in the males with autism but not in the control males. Zikopoulos and Barbas (2010) examined changes in axons in postmortem human brain tissue below the anterior cingulate cortex (ACC), orbitofrontal cortex (OFC), and lateral prefrontal cortex (LPFC) in autism and found a decrease in the largest axons that communicate over long distances and an excessive number of thin axons that link neighboring areas.

Numerous studies have reported abnormal connectivity in those with ASD (Wan and Schlaug 2010). Several terms are used to describe this impaired neuron connectivity found in those with autism, such as "underconnectivity", "impaired connectivity", "disrupted connectivity", or "altered brain connectivity" (Belmonte et al. 2004, Wan and Schlaug 2010, Wass 2011). More specifically, studies have reported underconnectivity in long-range connections and overconnectivity in short-range or local networks, with the frontal and temporal lobes being the most affected (Wass 2011). Examples of studies that found abnormal connectivity (both long-range underconnectivity and short-range overconnectivity) in those with autism are as follows:

Barttfeld and coauthors (2011) used electroencephalography (EEG) to assess dynamic brain connectivity in ASD focusing in the low-frequency (delta) range and found that those with ASD lacked long-range connections and had increased short-range connections. Interestingly, as ASD severity increased, short-range coherence was more pronounced and long-range coherence decreased. Using magnetoencephalographic (MEG), Pollonini and colleagues (2010) analyzed brain connectivity based on Granger causality computed from activity in eight subjects with autism and eight normal individuals. They found measurable connectivity differences between the two groups.

The cortical underconnectivity theory in autism was investigated by examining the neural bases of the visuospatial processing in one study in high-functioning autism. Using a combination of behavioral, functional magnetic resonance imaging, functional connectivity, and corpus callosum morphometric methodological tools, Damarla and coauthors (2010) found that the autism group had lower functional connectivity between the higher-order working memory/executive areas and the visuospatial regions (between frontal and parietal-occipital).

Several other studies using various methods, such as magnetic resonance imaging (MRI) and white matter parcellation technique, have examined connectivity in autism. These studies have all shown that functional

connectivity among regions of autistic brains is diminished (Herbert et al. 2004, 2005, Herbert 2005). For example, Ebisch and colleagues (2011), using functional magnetic resonance imaging (fMRI), found reduced functional connectivity in ASD, compared with controls, between anterior and posterior insula and specific brain regions involved in emotional and sensory processing. They stated that the functional abnormalities in a network involved in emotional and interoceptive awareness might be at the basis of altered emotional experiences and impaired social abilities in ASD.

For local, or short-range, overconnectivity, several studies have suggested regional brain overgrowth in autism. Stiglerand coauthors (2011) conducted a review of relevant structural and functional MRI studies in ASDs and reported "early rapid brain overgrowth in affected individuals". Similarly, from their imaging work, Courchesne and colleagues (2003) reported evidence of brain overgrowth in the first year of life in those with an autism diagnosis. They also reported that the excessive growth is followed by abnormally slow or arrested growth (Courchesne 2004). Again, Santos and coauthors (2010) found that the overgrowth clearly begins before 2 years of age. They conducted postmortem analysis of the brains of four young patients with autism and three controls of comparable age and found neuronal overgrowth in those with autism. Schumann and coauthors (2010) conducted a structural magnetic resonance imaging (MRI) longitudinal study of brain growth in toddlers at the time symptoms of autism are becoming clinically apparent and at multiple times thereafter (1.5 years up to 5 years of age), and found both cerebral gray and white matter were significantly enlarged in toddlers with ASD, with the most severe enlargement occurring in frontal, temporal, and cingulate cortices. The amygdale has also shown overgrowth. Measuring amygdala volumes on magnetic resonance imaging scans from 89 toddlers who were 1–5 years of age (mean = 3 years), Schumann and colleagues (2009) found overgrowth beginning before 3 years of age. Similar to the Barttfeld and coauthors (2011) study mentioned earlier, the extent of abnormal connectivity was associated with the severity of clinical impairments.

There are many more studies that suggest problems with connectivity in those with an ASD diagnosis. For a complete review of connectivity and list of the current studies that suggest abnormal brain connectivity in ASD, please see Wass (2011). As Wass (2011) observes in this recent review of connectivity in ASD, there is "considerable convergent evidence suggesting that connectivity is disrupted in ASD." From his review of the literature, he states that the evidence indicates both local over-connectivity and long-distance under-connectivity, and that disruptions appear more severe in the later-developing cortical regions. Long-range underconnectivity between regions and short-range over-connectivity appears to be pervasive in those with autism (Wass 2011). How Hg could cause long-range underconnectivity between regions and short-range over-connectivity in autism is discussed further in following section.

How Hg exposure could result in both the long-distance under-connectivity and local over-connectivity seen in autism

To date, there are only theories as to the cause of the short-range overconnectivity in ASD (Courchesne et al. 2011). This section will discuss how short-range overconnectivity could result from the loss of long-range axons.

As mentioned earlier, mercury seems to preferentially target large axons and to cause retraction/degeneration of those axons (Mitchell and Gallagher 1980, Stankovic 2006), and the evidence in autism shows axonal retraction, a decrease in the proportion of largest axons – the ones that communicate over long distances (Zikopoulos and Barbas 2010, Wass 2011). Since Hg causes the loss of long-range connectivity from degeneration of large, long-range axons, it is conceivable that the local outgrowth of axonal sprouting and dendritic overgrowth is a compensatory mechanism. The following evidence explains this model.

Following traumatic injury to central nervous system (CNS) axons, axons undergo what is called regenerative sprouting. Regenerative sprouting is when an injured neuron attempts to reform an injured axon. However, it is usually referred to as "abortive sprouting" (Schwartz and Flanders 2006), because of the inability of injured axons to cross the lesion site, to elongate, and to undergo true axonal regeneration (Meyer et al. 2009). The shorter the distance between the regeneration site and its distal target, the more successful regeneration of a nerve is likely to be, because postnatal, mature neuronal axons will only regenerate for very short distances in the CNS (Fawcett 1992).

Although many CNS neurons can survive for years after injury, the injured axons fail to regenerate beyond the lesion site in children and adults (Glenn and Zhigang 2006). The lack of a regenerative response is due, in part, to the presence of inhibitory molecules such as myelin-derived proteins or chondroitin sulphate proteoglycans (Hill et al. 2001, Seira et al. 2010). In addition, glial cells in the CNS (both oligodendrocytes and astrocytes) at the site of injury produce inhibitory molecules that inhibit axonal regrowth (Fawcett 1997, Stichel and Muller 1998, Goldshmit et al. 2004). As mentioned earlier, the loss of long-range axons from Hg appears to result in a slight increase in numbers of small axons (Pamphlett and Png 1998).

Although axonal regeneration is limited, the dendritic response to neuronal or axonal injury is overgrowth, i.e. an overproduction of dendritic branches (Jones and Schallert 1992, Jones 1999). It has been shown that following damage to connected brain regions, the brain undergoes an adaptive response which includes reactive axonal sprouting and an overproduction of dendrites (Jones 1999). Moreover, even though the overgrowth of dendrites eventually undergoes pruning, the overgrowth remains increased relative to controls (Jones and Schallert 1994, Jones 1999). Several other studies show that dentritic overgrowth secondary to neuronal injury is followed only by a partial reduction in the dendritic branching (Jones and Schallert 1992, Kozlowski et al. 1996, Brown and Murphy 2008).

Interestingly, in autism specifically, Zikopoulos and Barbas (2010) found a higher density of small axons and a significantly higher percentage of axons with branches compared to control cases, and that most points of bifurcation were unmyelinated or arose after thinning of the myelin. In patients with Minamata disease, caused from methylmercury poisoning (where the putative source of mercury compound was methylmercury cysteine – MeHgCys– from fish), regenerated axons were extremely small in size following regenerative sprouting and many fibers were found to be unmyelinated and poorly myelinated (Takeuchi et al. 1978).

Section summary statement

Mercury exposure can result in loss of long-range axons andlong-range underconnectivity and compensatory dendritic and axonal sprouting/short-range overgrowth. The deficit of long-range connectivity and short-range overconnectivity is what is found in the brain of those with ASD.

The evidence in this section suggests neuronal injury. Neuronal injury in the CNS would result in microglial activation. The next section discusses the evidence for microglia activation and neuroinflammation in the brain.

MICROGLIA ACTIVATION AND NEUROINFLAMMATION

Evidence of microglia activation, neuroinflammation, gliosis, and immune response in the CNS from Hg exposure

The brain responds to injury by rapidly activating the brain's own immune system, largely composed of glial cells (Streit et al. 2004, Streit and Xue 2009). Reactive gliosis specifically refers to the accumulation of enlarged glial cells (microglia and astrocytes) appearing immediately after a CNS injury has occurred (Vajda 2002). The presence of gliosis is suggestive of brain insult and neuroinflammation (Vajda 2002).

Microglia are the smallest of the glial cells and constitute approximately 20% of the glial cell population. Microglia are the resident macrophages of the central nervous system (CNS). They are considered to be the main form of immune defense in the CNS and are important for maintaining homeostasis. As key cellular mediators of the neuroinflammatory processes, microglia are associated with the pathogenesis of many neurodegenerative and brain inflammatory diseases (Ginhoux et al. 2010), and are involved in acute and chronic neuroinflammation (Streit et al. 2004, Streit and Xue 2009).

Once activated, microglia release nitric oxide (NO) and superoxide as a cytotoxic attack mechanism (Colton and Gilbert 1993). Reactive oxygen and nitrogen species (ROS and RNS) derived from NO and superoxide may also cause local cellular damage by reacting with proteins, lipids and nucleic acids (Valko et al. 2007). In addition, production of NO following microglial activation causes a decline in cellular glutathione (GSH) levels, leading to brain oxidative damage (Moss and Bates 2001). According to Stichel and Muller (1998), astrocytes in the adult show a vigorous response to injury; they become hypertrophic, proliferative as they upregulate expression of glial

fibrillary acidic protein (GFAP), and form a dense network of glial processes both at and extending from the lesion site. Streit and coauthors (2004) state that in the case of chronic neuroinflammation, the cumulative ill effects of microglial and astrocytic activation can contribute to and expand the initial neurodestruction, thus maintaining and worsening the disease process through their actions. Evidence suggests that the collateral neural damage can involve loss of synaptic connections in the brain (Gehrmann et al. 1995).

Numerous studies have shown that mercury exposure causes microglial activation, gliosis, neuroinflammation, and immune response in the CNS (Castoldi et al. 2003, Zhang et al. 2011). Specific examples of these processes include:

Using cell cultures of different complexity, isolated microglia were found to be directly activated by non-cytotoxic MeHgCl treatment by Eskes and colleagues (2002). The authors stated that microglial cells react just after a neurotoxic insult. Moreover, the interaction between activated microglia and astrocytes can increase local IL-6 release, which may cause astrocyte reactivity and neuroprotection.

In mice, Fujimura and coauthors (2009) administrated 30 ppm of methylmercury (MeHg) in drinking water for 8 weeks. They found a decrease in the number of neurons, an increase in the number of migratory astrocytes and microglia/macrophages, and necrosis and apoptosis in the cerebral cortex. In rats, Monnet-Tschudi and colleagues (1996) found a microglial response to long-term exposure to subclinical doses of Hg. These two studies suggest that the dose of Hg does not have to be high to get microglial activation. Again in rats, Gajkowska and colleagues (1992) administered a single dose (6 mg/kg body weight) of $HgCl_2$ to rats and found (after 18 hours) accumulation of dense deposits of mercury in nerve and glial cell cytoplasm with an increase in the quantity of microglia in the experimental group. Roda and coauthors (2008) investigated the effects of perinatal (GD7-PD21) administration of MeHg in drinking water (0.5 mg/kg bw/day) on cerebellum of immature (PD21) and mature (PD36) rats. They found reactive gliosis, e.g. a significant increase in Bergmann glia of the ML and astrocytes of the IGL, identified by their expression of glial fibrillary acidic protein. Vicente and coauthors (2004) also found glial involvement in the MeHg-induced neurotoxicity in rats.

Similarly, in monkeys, Charleston and colleagues (1996) examined effects of long-term subclinical exposure to methylmercury on microglia in the thalamus of the *Macaca fascicularis* and found microglia showed a significant increase in the 18-month and clearance exposure groups. Earlier, Charleston and coauthors (1995) examined effects of long-term subclinical exposure to methylmercury and mercuric chloride [$HgCl_2$, which directly releases Hg^{2+}(the putative inorganic mercury – IHg)] and found that the astrocytes and microglia in the MeHg exposure groups contained the largest deposits of IHg. They stated that all neurons in the 18-month exposure group contained deposits of IHg; however, these total deposits were considerably smaller than those within the astrocytes and microglia.

In humans, Eto and colleagues (1999) found changes produced by organic mercury in the brain of patients with Minamata disease who had acute onset of symptoms, and those who died within 2 months; they showed loss of neurons with reactive proliferation of glial cells. Interestingly, histochemistry of the mercury revealed that inorganic mercury was present in the brain.

Numerous studies have also shown that mercury exposure results in an increase in glial fibrillary acidic protein (GFAP). GFAP is elevated in acute and chronic situations of nerve cell damage and a marker of astroglial activation (Ahlsen et al. 1993). Examples are as follows:

El-Fawal and coauthors (1996) examined serum autoantibodies (Ig) to neurotypic and gliotypic proteins, myelin basic protein (MBP) and glial fibrillary acid protein (GFAP) as markers of subclinical neurotoxicity from methyl mercury (MeHg). They found that MeHg resulted in increased GFAP in the cerebellum at 14 days and elevation of several of the autoantibodies tested. Using GFAP as a quantitative marker of neuronal injuries on the central nervous system, Toimela and Tähti (1995) found that staining with monoclonal antibody showed GFAP induction after methylmercury exposure.

In a five laboratory collaborative study, Elsner and colleagues (1988) evaluated the effects of methylmercury on the *in utero* rat pups by treating rat dams at days 6 to 9 of gestation. They examined behavioral outcomes and GFAP and S-100 protein concentration in the rat pups, and found dose-dependent effects with increased GFAP concentration in the cerebellar vermis, increased auditory startle amplitude, and other

behavioral outcomes. Moreover, the study showed comparable sensitivities for the behavioral testing battery and the neurochemical assays.

Numerous studies show the activation of microglia and signs of neuroinflammation, gliosis, and immune response from mercury exposure in the brain of mammals. The evidence for the same findings in autism is the topic of the next section.

Evidence of microglial activation, neuroinflammation, gliosis, and immune response in autism

Evidence suggests that children with autism suffer from an ongoing inflammatory process in different regions of the brain involving microglial activation (Enstrom et al. 2005, Vargas et al. 2005, Zimmerman et al. 2005, Morgan et al. 2010). Herbert (2005) pointed out that the autistic brain is not simply wired differently, but that neuroinflammation is a part of the pathology in autism.

Vargas and coauthors (2005), for example, examined brain tissue and cerebral spinal fluid (CSF) in those with autism. For the morphological studies, brain tissues from the cerebellum, midfrontal, and cingulate gyrus were obtained at autopsy from 11 patients with autism. Fresh-frozen tissues from seven patients and CSF from six living patients with autism were used for cytokine protein profiling. The authors found active neuroinflammatory process in the cerebral cortex, white matter, and notably in cerebellum of patients with autism, with marked activation of microglia and astroglia. The authors stated that the CSF showed a unique proinflammatory profile of cytokines. The authors stated that the pattern of cellular and protein findings suggests the brain's own immune system (not immune abnormalities from outside the brain) and that the neuroinflammatory process appears to be an ongoing and chronic mechanism of CNS dysfunction.

Morgan and colleagues (2010) examined the dorsolateral prefrontal cortex of male cases with autism (n=13) and control cases (n=9) and found microglial activation and increased microglial density in the dorsolateral prefrontal cortex in those with autism. They also noted process retraction and thickening, and extension of filopodia (small protrusions sent out from a migrating cell in the direction that it wants to move) from the processes. The authors stated that the microglia were markedly activated in 5 of 13 cases with autism, including 2 of 3 under age 6, and marginally activated in an additional 4 of 13 cases. The authors stated that because of its early presence, microglial activation may play a central role in the brain pathogenesis of autism.

Several studies have shown that GFAP levels are increased in autism. An autopsy report by Bailey and coauthors (1998), found that the Purkinje cell loss was sometimes accompanied by gliosis and an increase in GFAP. Laurence and Fatemi (2005) examined levels of GFAP in the frontal, parietal, and cerebellar cortices using age-matched autistic and control postmortem specimens. GFAP was significantly elevated in all three brain areas. The authors stated that the elevated GFAP confirms microglial and astroglial activation in autism and indicates gliosis, reactive injury, and perturbed neuronal migration processes. A study by Ahlsen and colleagues (1993) examined the levels of GFAP in the CSF of children with autism, and found their average GFAP was three times higher than it was in the control group. The authors stated that the results could implicate gliosis and unspecified brain damage in children with autism. Likewise, Rosengren and colleagues (1992) found GFAP levels in CSF in children with autism were higher than those in normal control children of the same age range. The authors stated that the high levels of GFAP in combination with normal S-100 protein concentrations in CSF indicate reactive astrogliosis in the CNS.

Fatemi and coauthors (2008) investigated whether two astrocytic markers, aquaporin 4 and connexin 43, are altered in Brodmann's Area 40 (BA40, parietal cortex), Brodmann's Area 9 (BA9, superior frontal cortex), and the cerebella of brains of subjects with autism and matched controls. The authors reported that the findings demonstrated significant changes in two astrocytic markers in the brains from subjects with autism.

Section summary statement

Mercury exposure can result in activation of the brain's immune system characterized by elevated microglial cells and astrocytes, which is also found in the brains of those with ASD. Although this reaction is unspecific and may be triggered by many factors, the lack of microglial activation in either mercury intoxication or autism would decrease the probability of such causal connection.

OXIDATIVE STRESS AND LIPID PEROXIDATION

Free radicals and other reactive oxygen species (ROS) are produced in all species. Any free radical involving oxygen can be referred to as ROS, e.g. nitric oxide (NO). Free radicals and other ROS are unstable atoms, molecules, or ions with unpaired electrons. They are harmful because the unpaired electron oxidatively reacts with other ions and molecules, or they "steal" an electron from other molecules to pair that electron. This produces disruption to other molecules and damage to cells (Gutman 2002). One of the main problems is that ROS "steal" electrons from lipid membranes (the cell membrane of most living organisms is made of a lipid bilayer). The oxidative degradation of the lipid membrane is referred to as lipid peroxidation. Lipid peroxidation results in loss of membrane integrity and fluidity, which ultimately leads to cell death (Esterbauer et al. 1991, Efe et al. 1999). ROS also react with proteins and nucleic acids which can lead to cell death *via* apoptosis or necrosis (Kannan and Jain 2000).

Under normal conditions, a dynamic equilibrium exists between the production of ROS and the antioxidant capacity of the cell (Stohs 1995, Granot and Kohen 2004). Normally, the ROS within the cells are neutralized by antioxidant defense mechanisms. Superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) are the primary enzymes involved in direct elimination of ROS, whereas glutathione reductase and glucose-6-phosphate dehydrogenase are secondary antioxidant enzymes, which help in maintaining a steady concentration of reduced glutathione (GSH) and NADPH necessary for optimal functioning of the primary antioxidant enzymes (Chance 1954, Maddipati and Marnett 1987, Vendemiale et al. 1999). GSH is the most important antioxidant for detoxification and is important for the elimination of environmental toxins. Oxidative stress occurs when there is an imbalance between free radicals and the ability to neutralize them (i.e., an excess of pro-oxidants, a decrease in antioxidant levels, or both).

The brain is highly vulnerable to oxidative stress due to its limited antioxidant capacity, higher energy requirement, and higher amounts of lipids and iron (Juurlink and Paterson 1998). The brain makes up about 2% of body mass but consumes 20% of metabolic oxygen. Neurons use the vast majority of the body's energy (Shulman et al. 2004). Because neurons lack the capacity to produce GSH, the brain has a limited capacity to detoxify ROS. Therefore, neurons are the first cells to be affected by the increase in ROS and/or a shortage of antioxidants. As a result, they are susceptible to oxidative stress. Antioxidants are required for neuronal survival during the early critical developmental period (Perry et al. 2004). Children are more vulnerable than adults are to oxidative stress because of their naturally lower GSH levels from conception through infancy (Ono et al. 2001, Erden-Inal et al. 2002). The risk created by this natural deficit in detoxification capacity in infants is increased by the fact that some environmental factors that induce oxidative stress are found at higher concentrations in developing infants than in their mothers, and these preferentially accumulate in the placenta and the developing fetus. Taken together, these studies suggest that the developing brain is highly vulnerable to oxidative stress.

Evidence that Hg induces oxidative stress, lipid peroxidation, and altered glutathione levels and activity in the CNS

Numerous studies show that Hg exposure induces oxidative stress and lipid peroxidation in the CNS, as well as has a negative impact on glutathione and thiols (Ueha-Ishibashi et al. 2004, Huang et al. 2008, Monroe and Halvorsen 2009, Hoffman et al. 2011). Some relevant examples of pertinent studies include:

Huang and coauthors (2008) studied low-dose and long-term exposure of methylmercury (MeHg) in mice. They found significant Hg accumulation and biochemical alterations in brain regions and/or other tissues, including the increase of lipid peroxidation (LPO) production, influence of Na^+/K^+-ATPase activities and nitric oxide (NO) levels. Again in 2011, Huang and his colleagues examined the underlying mechanisms of neurotoxic effects of both methylmercury (MeHg) and mercury chloride ($HgCl_2$) in mice and found that the alteration of lipid peroxidation (LPO), Na^+/K^+-ATPase activities, and nitric oxide (NO_x) in the brain tissues contributed to the observed neurobehavioral dysfunction and hearing impairment (Huang et al. 2011).

Even studies that gave antioxidants in conjunction with mercury found oxidative stress in the brain. Glaser and coauthors (2010), for example, gave adult

male mice MeHg orally in drinking water (40 mg/ L^{-1}), and simultaneously administrated daily subcutaneous injections of sodium selenite (Na_2SeO_3). Although there was a reduction in cells with metal deposition in the brain, there was still an increase in lipid peroxidation in the brain.

Many studies that show mercury induces oxidative stress and lipid peroxidation in the brain also find a concomitant decrease in glutathione levels, as well as alterations in GSH-related enzymes (Ou et al. 1999, Manfroi et al. 2004, Franco et al. 2006, 2007, 2009, 2010, Stringari et al. 2006, 2008, Yin et al. 2007, Aschner et al. 2007). Franco and colleagues (2010), for example, found that incubation of mouse brain mitochondria with MeHg induced a significant decrease in mitochondrial function, which was correlated with decreased GSH levels and increased generation of ROS and lipid peroxidation.

As reported by Stringari and coauthors (2006), and other studies (Manfroi et al. 2004, Franco et al. 2006), the GSH antioxidant system is a significant molecular target of MeHg and during the early postnatal period, mercury exposure results in decreased GSH levels and decreased activities of GSH-related enzymes. Moreover, Stringari and colleagues (2008) found, in a follow up study, that mercury exposure effectively inhibited the developmental profile of the cerebral GSH antioxidant system during the early postnatal period. The authors went on to state that the inhibition of the maturation the GSH antioxidant system might contribute to the oxidative damage seen after prenatal MeHg exposure, because even though the cerebral mercury concentration decreased later postnatally, the GSH levels, glutathione peroxidase (GPx) and glutathione reductase (GR) activities remained decreased in MeHg-exposed mice. According to Stringari and colleagues (2008), the evidence corroborates previous reports that indicate prenatal exposure to MeHg affects the GSH antioxidant systems by inducing biochemical alterations that persist even when mercury tissue levels decreased to the same levels as those in the controls. This early exposure induces pro-oxidative damage and permanent functional deficits in the developing CNS.

Ueha-Ishibashi and coauthors (2004) examined the effects of Thimerosal (sodium ethylmercury thiosalicylate, Na^+ $EtHgSal^-$), an organomercurial preservative in vaccines, on cerebellar neurons dissociated from 2-week-old rats, as compared to methylmercury, and found that both agents (at 1 μM or more) similarly decreased the cellular content of glutathione in a concentration-dependent manner, suggesting an increase in oxidative stress. As evident in this study, it is important to note that many of the studies mentioned in this section show a dose-dependent affect, i.e., the greater the levels of Hg, the higher the levels of oxidative stress.

Evidence of oxidative stress, lipid peroxidation, and altered glutathione levels and activity in the brain in autism

Three *postmortem* studies published in 2008 revealed that affected areas of the brain in children with autism showed accelerated cell death under conditions of oxidative stress (Lopez-Hurtado and Prieto 2008, Evans et al. 2008, Sajdel-Sulkowska et al. 2008). Evans and coauthors (2008), for example, evaluated the oxidative stress metabolites of carboxyethyl pyrrole (CEP) and iso[4]levuglandin (iso[4]LGE_2-protein adducts in cortical brain tissues of subjects diagnosed with autism. Significant immunoreactivity toward these markers of oxidative damage in the white matter, often extending well into the grey matter of axons, was found in every case of autism examined. These investigators reported that the striking thread-like pattern appears to be a hallmark in the brains of those diagnosed with ASD, as it was not seen in any control brains, young or aged, used as controls for the oxidative assays. In another study, the density of lipofuscin, a matrix of oxidized lipid and cross-linked protein that forms as a result of oxidative injury in the tissues, was observed to be greater in cortical brain areas concerned with communication in subjects diagnosed with autism (Lopez-Hurtado and Prieto 2008) than in controls. Lipofuscin was previously demonstrated to be a depot for mercury in human brain autopsy specimens from mercury-intoxicated patients (Opitz et al. 1996). Finally, and perhaps most importantly, Sajdel-Sulkowska and colleagues (2008) evaluated cerebellar levels of the oxidative stress marker 3-nitrotyrosine (3-NT), mercury, and the antioxidant selenium in subjects diagnosed with autism and in control subjects. These researchers found that there were significant increases in the mean cerebellar levels of 3-NT and in the ratio of mercury/selenium in the brains of subjects diagnosed with autism when compared to controls. Importantly, there was a significant dose-dependent positive correlation between the oxidative stress markers and total mercury

levels. This dose-dependent effect is seen in many studies (as shown in the previous section) in animals and humans.

In 2009, Sajdel-Sulkowska and colleagues also published a study where they examined oxidative damage in the cerebellum of those with ASD by measuring 8-hydroxydeoxyguanosine (8-OH-dG), a marker of DNA modification, in a subset of cases they also analyzed for 3-NT. The authors found that cerebellar 8-OH-dG showed an upward trend toward higher levels with an increase of 63.4% observed in those with autism. Analysis of cerebellar neurotrophin-3 (NT-3) showed a statistically significant (P=0.034) increase (40.3%) in those with autism. Furthermore, there was a significant positive correlation between cerebellar NT-3 and 3-NT. The authors stated that the altered levels of brain NT-3 are likely to contribute to autistic pathology not only by affecting brain axonal targeting and synapse formation but also by further exacerbating oxidative stress and possibly contributing to Purkinje cell abnormalities.

Later in 2011, Sajdel-Sulkowska and coauthors examined whether the increase in oxidative stress in ASD is brain region-specific. They compared brain region-specific NT-3 expression between those with ASD and control cases. The 3-NT and NT-3 were measured with specific ELISAs in individual brain regions of two autistic and age- and postmortem interval (PMI)-matched control donors. The authors found that the levels of 3-NT, ranging from 1.6 to 12.0 pmol/g, were uniformly low in all brain regions examined in controls. However, there was a large degree of variation in 3-NT levels and its maximum levels were much higher, ranging from 1.7 to 281.2 pmol/g, among individual brain regions in those with autism. The brain regions with the increased 3-NT levels and the magnitudes of the increase were both different in the two autistic cases. In the brain of the older case, the brain regions with highest levels of 3-NT included the orbitofrontal cortex (214.5 pmol/g), Wernicke's area (171.7 pmol/g), cerebellar vermis (81.2 pmol/g), cerebellar hemisphere (37.2 pmol/g), and pons (13.6 pmol/g) (brain areas associated with the speech processing, sensory and motor coordination, emotional and social behavior, and memory). Brain regions that showed 3-NT increases in both of those with ASD included the cerebellar hemispheres and putamen. Consistent with their earlier report, the researchers found an increase in NT-3 levels in the cerebellar hemisphere in both of the brains from subjects who had been diagnosed with ASD. They also detected an increase in NT-3 level in the dorsolateral prefrontal cortex (BA46) in the brain from the older individual and in the Wernicke's area and cingulate gyrus in the brain of the younger case.

Many studies have shown that plasma GSH levels are low and biomarkers of oxidative stress are high in ASD (Geier et al. 2009c); moreover, in recent study by Chauhan and coauthors (2012), they found that the same is true when directly measuring brain tissue in ASD. They compared DNA oxidation and glutathione redox status in postmortem brain samples from the cerebellum and frontal, temporal, parietal and occipital cortex from autistic subjects and age-matched normal subjects. The authors reported that DNA oxidation was significantly increased by two-fold in frontal cortex, temporal cortex, and cerebellum in individuals with autism as compared with control subjects. Moreover, the levels of reduced glutathione GSH were significantly reduced and the levels of oxidized glutathione GSSG were significantly increased in the cerebellum and temporal cortex in the brain samples from the group with autism as compared to the corresponding levels in the control brain samples.

Earlier, Chauhan and colleagues (2011) studied the levels of mitochondrial electron transport chain (ETC) complexes in brain tissue samples from the cerebellum and the frontal, parietal, occipital, and temporal cortices of subjects with autism and age-matched control subjects. The subjects were divided into two groups according to their ages: Group A (children, ages 4–10 years) and Group B ("adults", ages 14–39 years). A significant increase in the levels of lipid hydroperoxides, an oxidative stress marker, was observed in the cerebellum and temporal cortex in the children with autism as compared to the levels in the controls. The authors also found evidence of mitochondrial dysfunction in the brain, which will be discussed further in the following section.

Section summary statement

Mercury intoxication can result in elevated oxidative stress markers and lowered GSH levels in the brain. Both are present in the brains of persons with ASD.

MITOCHONDRIAL DYSFUNCTION

Evidence of mitochondrial damage and dysfunction in the brain from Hg exposure

Numerous studies show that Hg causes systemic mitochondrial dysfunction (Stohs and Bagchi 1995, Stacchiotti et al. 2010, Belyaeva et al. 2011) and causes mitochondrial dysfunction in the brain (Stohs and Bagchi 1995, Allen et al. 2001, Castoldi et al. 2003, Limke et al. 2004, Yin et al. 2007, Dreiem and Seegal 2007, Franco et al. 2007, 2010, Monroe and Halvorsen 2009, Kaur et al. 2010, Migdal et al. 2010). Although mercury, as stated by Yin and coauthors (2007), "initiates multiple additive or synergistic disruptive mechanisms," the main mechanism of disruption to mitochondrial function appears to result from the mercury-induced production of ROS. As mentioned before, Franco and colleagues (2010) found that incubation of mouse brain mitochondria with MeHg induced a significant decrease in mitochondrial function, which was correlated with decreased GSH levels and increased generation of ROS and lipid peroxidation. Hg depletes GSH and protein-bound sulfhydryl groups, resulting in the production of ROS, and as a consequence, lipid peroxidation, and specifically mitochondrial lipid peroxidation occurs (Stohs and Bagchi 1995, Kaur et al. 2010). As mentioned in a previous section, lipid peroxidation results in membrane permeability. Yin and colleagues (2007), for example, found that methylmercury exposure results in a concentration-dependant reduction in the inner mitochondrial membrane potential and increased mitochondrial membrane permeability. Further, Migdal and coauthors (2010) found that Thimerosal, a mercury derivative composed of ethyl mercury chloride (EtHgCl) and thiosalicylic acid (TSA), caused mitochondrial membrane depolarization and changes in mitochondrial membrane permeability. In addition, methylmercury induces the overproduction of hydrogen peroxide (H_2O_2), which in turn, inhibits astrocyte glutamate transporters, and leads to increased glutamate concentrations and glutamate-induced oxidative stress. Thus, both direct Hg-induced oxidative stress and glutamate-induced oxidative stress result in mitochondrial dysfunction (Allen et al. 2001, Franco et al. 2007).

Although several studies have found that Hg induces mitochondrial dysfunction secondary to the formation ROS, Dreiem and Seegal (2007) found that even when mitochondria are exposed to methylmercury chloride in conjunction with the antioxidant Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, a water-soluble derivative of vitamin E), which significantly reduced MeHgCl-induced ROS levels, it failed to restore mitochondrial function. The authors found that mercury also increased mitochondrial calcium levels in striatal synaptosomes, and proposed that the increased mitochondrial calcium levels also contribute to the mitochondrial dysfunction. In other words, the MeHgCl disrupted calcium homeostasis critical for mitochondrial function.

The following section discusses the evidence for mitochondrial dysfunction in the brains of those with ASD.

Evidence of mitochondrial damage and dysfunction in the brain in autism

A recent review by Palmieri and Persico (2010), reports that a substantial percentage of patients with ASD display peripheral markers of mitochondrial energy metabolism dysfunction, such as (a) elevated lactate, pyruvate, and alanine levels in blood, urine and/or cerebrospinal fluid, (b) serum carnitine deficiency, and/or (c) enhanced oxidative stress. Other researchers have also reported evidence of systemic mitochondrial dysfunction (Giulivi et al. 2010). Moreover, recent evidence from two postmortem studies of the brains of those with ASD points specifically toward abnormalities in mitochondrial function in the brain. Two examples are as follows:

First, Chauhan and colleagues (2011) studied the levels of mitochondrial electron transport chain (ETC) complexes, i.e. complexes I, II, III, IV, and V, in brain tissue samples from the cerebellum and the frontal, parietal, occipital, and temporal cortices of subjects with autism and age-matched control subjects. The subjects were divided into two groups according to their ages: Group A (children, ages 4–10 years) and Group B ("adults", ages 14–39 years). In Group A, they observed significantly lower levels: of complexes III and V in the cerebellum ($P<0.05$), of complex I in the frontal cortex ($P<0.05$), and of complexes II ($P<0.01$), III ($P<0.01$), and V ($P<0.05$) in the temporal cortex of children with autism as compared to age-matched control subjects. In the cerebellum and temporal cortex, no overlap was observed in the levels of these ETC complexes between subjects with autism and the con-

trol subjects. In the frontal cortex of Group A, a lower level of ETC complexes was observed in a subset of autism cases, i.e. 60% (3/5) for complexes I, II, and V, and 40% (2/5) for complexes III and IV. A striking observation, made by the authors, was that the levels of ETC complexes were similar in adult subjects with autism and the control subjects (Group B). A significant increase in the levels of lipid hydroperoxides, an oxidative stress marker, was also observed in the cerebellum and temporal cortex in the children with autism. These results suggest that the expression of ETC complexes is decreased in the cerebellum and the frontal and temporal regions of the brain in children with autism, which may lead to abnormal energy metabolism and oxidative stress. Based on these findings, the significant deficits observed in the levels of ETC complexes in children with autism may readjust to normal levels by adulthood.

Second, Palmieri and coauthors (2010) studied mitochondrial dysfunction in autism using temporocortical gray matter from six matched patient-control pairs. The authors performed postmortem biochemical and genetic studies of the mitochondrial aspartate/glutamate carrier (AGC), which participates in the aspartate/malate reduced nicotinamide adenine dinucleotide shuttle and is physiologically activated by calcium (Ca^{2+}). They found that the AGC transport rates were significantly higher in tissue homogenates from all six patients, including those with no history of seizures and with normal electroencephalograms prior to death. The increase was consistently blunted by the Ca^{2+} chelator ethylene glycol tetraacetic acid. In addition, neocortical Ca^{2+} levels were significantly higher in all six patients, and no difference in AGC transport rates was found in isolated mitochondria from patients and controls following removal of the Ca^{2+}-containing postmitochondrial supernatant. Expression of AGC1, the predominant AGC isoform in brain, and cytochrome c oxidase activity were both increased in the patients diagnosed with ASD, indicating an activation of mitochondrial metabolism. Furthermore, oxidized mitochondrial proteins were markedly increased in four of the six study patients. The authors stated that excessive Ca^{2+} levels are responsible for boosting AGC activity, mitochondrial metabolism and, to a more variable degree, oxidative stress in the brains of those with ASD.

As mentioned previously, mercury disrupts calcium homeostasis in the CNS, and as just mentioned excessive Ca^{2+} levels are involved in the mitochondrial dysfunction in autism. The following section provides more evidence for Ca^{2+} disruption as a product of mercury exposure and evidence of this disruption in the brains of those with ASD.

Section summary statement

Mercury intoxication can result in mitochondrial dysfunctionin the brain involving increased mitochondrial levels of calcium, which is what is found in the brains of those with ASD.

CALCIUM HOMEOSTASIS

Evidence of disruption in calcium homeostasis and signaling in the CNS from Hg exposure

Calcium is involved in several activities in the CNS such as transmitter release, long-term potentiation, growth cone motility, ion channel inactivation, neuronal growth, differentiation, motility and excitability, secretion of neurotransmitters and hormones, synaptic plasticity, neurotoxicity and neuronal gene expression, and calcium signaling (Cote and Crutcher 1991, Lohmann 2009, Lozac 2010). Optimum levels of calcium influx promote normal dendritic and axonal elongation and growth cone movements (Lozac 2010). One reason that calcium homeostasis is critical in the CNS is because an imbalance of calcium, such as an excessive influx of Ca^{2+} ions into the neuron, can lead to inflammation, free radicals, and ultimately cause neuronal cell death (Cote and Crutcher 1991). Calcium signaling (i.e. signal transduction mechanisms where calcium mobilization, from outside the cell or from intracellular storage pools, to the cytoplasm is brought about by external stimuli) is important because it directs structural and functional adaptations in neurons that underlie the establishment of synaptic specificity (Lohmann 2009).

Mercury-induced neurotoxicity includes impairment of intracellular calcium homeostasis (Sirois and Atchison 2000, Castoldi et al. 2003, Atchison 2005, Ceccatelli et al. 2010) and calcium signaling (Limke et al. 2004). Calcium homeostasis is disrupted mainly because mercury induces Ca^{2+} increases *via* Ca^{2+} influx from the extracellular space (Olanow and Arendash 1994, Liu et al. 2007). Examples of evidence are as follows.

Marty and Atchison (1997) used cell imaging and the Ca^{2+}-sensitive fluorophore fura-2 to investigate the methylmercury's effect on Ca^{2+} homeostasis in rat cerebellar granule cells, that are preferentially targeted by methylmercury. *In vitro* methylmercury exposure (0.2–5.0 μM) induced a biphasic rise in fura-2 fluorescence ratio, consisting of a small first phase due to Ca^{2+} release from intracellular stores and a much larger second phase which required Ca^{2+} influx to the cell. As mentioned in an earlier section on oxidative stress, Gassó and coauthors (2001) studied the involvement of oxidative stress and Ca^{2+} homeostasis disruption in Hg-induced cytotoxicity on rat cerebellar granule neuron cultures using MeHgCl and $HgCl_2$, and found that, after 24 hours of exposure, there was a Hg-mediated (Ca^{2+}) rise. The results of the study indicated that Ca^{2+} influx through Ca^{2+} channels and the Na^+/Ca^{2+}exchanger and Ca^{2+}mobilization from the endoplasmic reticulum are involved in mercury-mediated cytotoxicity. The authors stated that disruption of redox equilibrium and Ca^{2+}homeostasis contribute equally to $HgCl_2$-mediated toxicity, whereas oxidative stress is the main cause of MeHg neurotoxicity.

In order to examine the effects of Thimerosal (which rapidly converts to a mixture of ethylmercury chloride, ethylmercury hydroxide, and sodium thiosalicylates in isotonic saline-based biological media), Zieminska and colleagues (2010) studied neuron viability, intracellular levels of calcium and zinc, as well as mitochondrial membrane potential in primary cultures of rat cerebellar granule cells. The study found that Thimerosal evoked: (1) a decrease in the cells viability, (2) a rise in the intracellular calcium and zinc concentration, and (3) decrease in mitochondrial membrane potential.

Ueha-Ishibashi and coauthors (2004) examined the effects of Thimerosal as compared to methylmercury on cerebellar neurons dissociated from 2-week-old rats. Thimerosal and methylmercury at concentrations ranging from 0.3 to 10 μM increased the intracellular concentration of Ca^{2+}[Ca^{2+}(i)] in a concentration-dependent manner. The authors stated that the cytotoxic potency of Thimerosal and methylmercury were similar. All of these aforementioned studies on rat cerebellar neurons clearly show that several forms of mercury have detrimental effects on calcium homeostatis in cerebellar neurons.

Limke and colleagues (2004) conducted a review of the mechanisms underlying the specific targeting of cells during MeHg poisoning, and stated that the disruption of [Ca^{2+}](i) regulation occurs through specific pathways which affect Ca^{2+} regulation by organelles, particularly mitochondria and the smooth endoplasmic reticulum (SER). Cholinergic pathways which affect [Ca^{2+}](i) signaling also appear to be critical targets, particularly muscarinic acetylcholine (ACh) receptors which are linked to Ca^{2+} release through inositol-1,4,5-triphosphate [IP(3)] receptors. [Ca^{2+}](i) dysregulation may also underlie observed alterations in cerebellar neuron development through interaction with specific target(s) in the developing axon. However, Atchison (2005) studied MeHg and stated that his research suggested that it is the mercury-induced release of glutamate, which, coupled with a Hg-induced impairment of glutamate uptake by astrocytes, could also cause the Ca^{2+}-mediated cytotoxicity.

Sirois and Atchison (2000) tested the ability of methylmercury (MeHg) to block calcium channel current in cultures of neonatal cerebellar granule cells using whole-cell patch clamp techniques and Ba^{2+} as charge carrier. The results showed that acute exposure to submicromolar concentrations of MeHg can block Ba^{2+} currents carried through multiple Ca^{2+} channel subtypes in primary cultures of cerebellar granule cells.

As seen, many studies show mercury neurotoxicity includes altered calcium homeostasis. Evidence also suggests that calcium homeostasis is altered in autism, which is the topic of the next section.

Evidence of disruption in calcium homeostasis and signaling in the CNS in autism

Palmieri and Persico (2010) stated in their review mentioned earlier that recent evidence from postmortem studies of the brains of those with ASD suggests abnormalities in mitochondrial function as possible downstream consequences of dysreactive immunity and altered Ca^{2+} signaling. Although the topic is new and the research just beginning, there is a study mentioned earlier in the section on mitochondria that shows evidence of altered calcium homeostasis in autism (Palmieri et al. 2010). Palmieri and coauthors (2010) performed postmortem biochemical and genetic studies of the temporocortical gray matter from six matched patient-control pairs to examine the mitochondrial aspartate/glutamate carrier (AGC), which participates in the aspartate/malate reduced nicotinamide adenine dinucleotide shuttle and is physiologi-

cally activated by Ca^{2+}. They found that excessive Ca^{2+} levels are responsible for boosting AGC activity, mitochondrial metabolism and, to a more variable degree, oxidative stress in autistic brains. The authors stated that AGC and altered Ca^{2+} homeostasis play a key interactive role in the cascade of signaling events leading to autism.

Section summary statement

Mercury intoxication can result in altered Ca^{2+} homeostasis, involving increased or excessive calcium levels, which is also what is found in the brains of those with ASD.

GABA-ERGIC AND GLUTAMATERGIC HOMEOSTASIS

Evidence of disruption of GABAergic and glutamatergic homeostasis from Hg exposure

Glutamate is the most common neurotransmitter in the brain, and it is excitatory. Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter of the brain, second only to glutamate as a major brain neurotransmitter. In order to maintain normal functioning of the brain, glutamate and GABA homeostasis is critical, especially glutamate homeostasis, because an excess of excitatory neurotransmitters can cause excitotoxicity and neuronal cell death (Mutkus et al. 2005).

Many studies provide evidence that Hg-induced neurotoxicity includes disruptions in the GABA (GABAergic) and glutamate (glutamatergic) systems in the CNS (Arakawa et al. 1991, Soares et al. 2003, Castoldi et al. 2003, Mutkus et al. 2005, Basu et al. 2007, van Vliet et al. 2007). Some examples are as follows:

Beginning with GABA, evidence suggests that one of the main neurotoxic effects of Hg is due to the inhibition of the rate-limiting GABA synthesizing enzyme, glutamic acid decarboxylase (GAD). Because GAD is the rate-limiting enzyme responsible for normal conversion of glutamate to GABA in the brain, measuring GAD activity can be used to determine any underlying abnormalities in the GABAergic system (Fatemi et al. 2002, 2009, 2010, Yin et al. 2007). Basu and colleagues (2010), for example, examined GAD activity and found that Hg disrupts GABAergic systems in discrete brain regions in captive mink. Importantly, they found that *in vitro* studies on cortical brain tissues revealed that inorganic Hg ($HgCl_2$) and methylmercurychloride (MeHgCl) inhibited glutamic acid decarboxylase (GAD) activity. The authors stated that the results show that chronic exposure to environmentally relevant levels of MeHg disrupts GABAergic signaling.

There are several other ways that mercury induces alterations in GABA homeostasis. Some examples are as follows:

Fonfría and coauthors (2001) found that mercury interacts with the GABA(A) receptor by the way of alkylation of SH groups of cysteinyl residues found in GABA(A) receptor subunit sequences in cerebellar granule cells. Hogberg and colleagues (2010) found a significant downregulation of the GABA receptor rat cerebellar granule cells after exposure to methylmercury chloride.

Rubakhin and colleagues (1995) examined the effects of inorganic Hg ($HgCl_2$) on GABA activated Cl^- currents on Lymnaea neurons. They found that Hg enhanced GABA-evoked Cl^- permeability and the higher the Hg concentrations, the higher the membrane permeability.

Narahashi and coauthors (1994) examined the GABA(A) receptor-chloride channel complex and found that it is a target site of Hg. Kumamoto and colleagues (1986) found that MeHgCl greatly inhibits the GABA uptake into the satellite cells in the dorsal root ganglia.

Huang and Narahashi (1997) found $HgCl_2$ potentiation of GABA-induced currents. Importantly, they showed that $HgCl_2$ potentiation of GABA-induced currents was use dependent, increasing with the frequency of channel openings. However, the potentiation was blocked by cysteine and iodoacetamide suggesting involvement of sulfhydryl groups in this action (Huang and Narahashi 1996). As mentioned previously, ionic and alkyl Hg have a strong affinity for sulfhydryl groups.

Similar to GABA research, many studies report Hg induced alterations of glutamate homeostasis (Farina et al. 2003a,b, Aschner et al. 2000, Mutkus et al. 2005). Examples are as follows:

In a recent analysis of the effects of Hg on the glutamatergic system, Aschner and coauthors (2000) for example, found that MeHg-induced dysregulation of excitatory amino acid homeostasis. They stated that

MeHg induces swelling of astrocytes and specifically inhibits glutamate uptake in astrocytes. This finding was also noted by several others (Brookes and Kristt 1989, Danbolt 2001, Castoldi et al. 2003, Morken et al. 2005).

Yin and colleagues (2011) found that MeHg treatment significantly decreased (P<0.05) astrocytic [(3) H]-glutamine uptake at all time points and concentrations. The authors stated that the MeHg-induced changes in astrocytic [(3)H]-glutamine uptake also resulted in dissipation of the astrocytic mitochondrial membrane potential.

In addition, MeHg increases glutamate extracellular levels (Juárez et al. 2002, 2005), increases spontaneous glutamate release (Reynolds and Racz 1987), and brings about inhibition of glutamate net uptake. These changes also appear to be related to the cells ability in maintain cell viability (Moretto et al. 2005, Morken et al. 2005). Likewise, Vendrell and coauthors (2007) reported that methylmercury produces loss of cell viability, reduced intracellular glutamate content, and increased lipid peroxidation in cultured cerebellar granule cells of mice.

Soares and colleagues (2003) stated that Hg induces [3H]-glutamate binding inhibition. Importantly, studies have found a significant decrease in N-methyl-D-aspartate (NMDA) receptor levels following Hg exposure (Basu et al. 2007, 2009, Adams et al. 2010). The NMDA receptor is a specific type of ionotropic glutamate receptor.

Other examples of studies that show a significant decrease in NMDA receptor levels following Hg exposure are as follows:

Wyrembek and colleagues (2010), for example, examined Thimerosal and $HgCl_2$ effects on GABA and NMDA-evoked currents in cultured hippocampal neurons using electrophysiological recordings. Following exposure for 60–90 min to 1 or 10 μM Thimerosal, there was a significant decrease in NMDA-induced currents (P<0.05) and GABAergic currents (P<0.05). Thimerosal was also neurotoxic, damaging a significant proportion of neurons after 60–90 min of exposure (recordings were always conducted in the healthiest looking neurons). $HgCl_2$, at concentrations 1 μM and above, was even more toxic, killing a large proportion of cells after just a few minutes of exposure. Recordings from a few sturdy cells revealed that micromolar $HgCl_2$ markedly potentiated the GABAergic currents (P<0.05), but reduced NMDA-evoked currents (P<0.05). The authors stated $HgCl_2$ act rapidly, decreasing electrophysiological responses to NMDA but enhancing responses to GABA, while Thimerosal works slowly, reducing both NMDA and GABA responses. The neurotoxic effects of both mercurials are interwoven with their modulatory actions on GABA(A) and NMDA receptors, which most likely involve binding to these macromolecules.

In addition, Mutkus and coauthors (2005) showed that MeHg selectively affects glutamate transporter mRNA expression. Glutamate transporters provide glutamate for synthesis of GABA and glutathione (Danbolt 2001).

As noted in this section, Hg neurotoxicity includes disruption of the glutamate and GABA systems. These systems are found to be aberrant in autism, which is the topic of the next section.

Evidence of alteration of GABAergic and Glutamatergic homeostasis in autism

Many studies have demonstrated abnormalities involving the GABAergic and glutamatergic systems in the autistic brain (Blatt et al. 2001, Dhossche et al. 2002, Fatemi et al. 2008, 2009, Fatemi 2009). Fatemi and colleagues (2002, 2009, 2010), for example, conducted several studies on the topic of altered GABA homeostasis in autism. These studies are as follows:

First, they demonstrated that brain levels of glutamic acid decarboxylase 65 and 67kDa proteins (GAD65/67) were significantly decreased in cerebellum (GAD65) and parietal cortex in autism (Fatemi et al. 2002). As mentioned, GAD is the rate limiting enzyme responsible for normal conversion of glutamate to GABA in the brain. This finding was also reported by Yip and coauthors (2009), who found a mean 51% reduction in GAD65 mRNA levels in the larger labeled cells in the autistic group compared with the levels in the control group (P=0.009) but not in the smaller cell subpopulation. And earlier in 2007, Yip and colleagues reported that GAD67 mRNA level was reduced by 40% in the autistic group, consistent with previous reports of alterations in the GABAergic system in limbic and cerebro-cortical areas in the brains of those diagnosed with ASD.

Second, Fatemi and coauthors (2009) investigated the expression of four GABA(A) receptor subunits and observed significant reductions in GABRA1, GABRA2, GABRA3, and GABRB3 in parietal cortex

[Brodmann's Area 40 (BA40)], while GABRA1 and GABRB3 were significantly altered in cerebellum, and GABRA1 was significantly altered in superior frontal cortex (BA9). Their results demonstrate that GABA(A) receptors are reduced in three brain regions that have previously been implicated in the pathogenesis of autism, suggesting widespread GABAergic dysfunction in the brains of subjects with autism. Blatt and coauthors (2001) also found that GABA(A) receptors are significantly reduced in autism.

Third, Fatemi and colleagues (2010) sought to verify their western blotting data for GABBR1 *via* qRT-PCR and to expand their previous work to measure mRNA and protein levels of 3 GABA(A) subunits previously associated with autism (GABRα4; GABRα5; GABRβ1). Three GABA receptor subunits demonstrated mRNA and protein level concordance in superior frontal cortex (GABRα4, GABRα5, GABRβ1) and one demonstrated concordance in cerebellum (GABBR1). These results provide further evidence of impairment of GABAergic signaling in autism.

In addition, Harada and coauthors (2011) examined the neurotransmitters in the GABAergic/glutamatergic system in 12 patients with autism and 10 normal controls and found that the [GABA]/[Glu] ratio was significantly lower ($P<0.05$) in the patients with autism than in the normal controls. The authors stated that this finding thus suggested a possible abnormality in the regulation between GABA and glutamate.

Similar to GABA, several more studies suggest disruptions in glutamate homeostasis in ASD (O'Neill et al. 2003). Bernardi and colleagues (2011), for example, investigated cellular neurochemistry with proton magnetic resonance spectroscopy imaging [(1)H-MRS] in brain regions associated with networks subserving alerting, orienting, and executive control of attention in patients with ASD. The ASD group showed significantly lower glutamate concentration in right anterior cingulate cortex. Page and coauthors (2006) also used proton magnetic resonance spectroscopy [(1)H-MRS] to measure the concentration of brain metabolites of the amygdala-hippocampal complex and a parietal control region in adults with ASDs and in healthy subjects. Patients with ASD had a significantly higher concentration of glutamate/glutamine and creatine/phosphocreatine in the amygdala-hippocampal region but not in the parietal region.

DeVito and coauthors (2007) examined cerebral gray and white matter cellular neurochemistry in autism with proton magnetic resonance spectroscopic imaging (MRSI) in 26 males with autism (age 9.8 ± 3.2 years) and 29 male comparison subjects (age 11.1 ± 2.4 years). They found that patients with autism exhibited significantly lower levels of gray matter glutamate than the control subjects. The deficits were widespread, according to the authors, affecting most cerebral lobes and the cerebellum. The authors stated that these results suggest widespread reductions in gray matter neuronal integrity and dysfunction of cortical and cerebellar glutamatergic neurons in patients with autism.

Purcell and colleagues (2001) examined brain samples from a total of 10 individuals with autism and 23 matched controls, mainly from the cerebellum. The authors reported that the finding showed evidence for specific abnormalities in the AMPA-type glutamate receptors and glutamate transporters in the cerebellum. Corroborating these brain studies which suggest disrupted GABA and glutamate homeostasis, GABA and glutamate levels are found to be elevated in the plasma in autism as compared to controls (Dhossche et al. 2002, Shinohe et al. 2006).

Section summary statement

Mercury intoxication can result in altered of GABAergic and glutamatergic homeostasis, involving various areas of the brain, which is also found in the brains in those with ASD.

INSULIN-LIKE GROWTH FACTOR 1 (IGF-1) SIGNALING, METHIONINE SYNTHASE INHIBITION, METHYLATION

Evidence of disruption in insulin-like growth factor 1 (IGF-1) signaling and methionine synthase inhibition resulting in adverse effects on methylation from Hg exposure

Insulin-like growth factor 1 (IGF-1) is a protein hormone similar in molecular structure to insulin. IGF-1 has also been referred to as a "sulfation factor" (Salmon and Daughaday 1957). It is important in childhood growth and continues to have effects in adults. Evidence suggests that IGF-1 in the brain has neuroprotective effects (Tang et al. 2011). For instance, Park and coauthors (2011) found that IGF-1 down regulates glial activation and induces expression of an endogenous growth factor.

Methionine synthase is an enzyme that interacts with vitamin B-12 and folate to regenerate the amino acid methionine from homocysteine (Gallagher and Meliker 2011). When methionine synthase activity is inhibited, this in turn, inhibits methylation reactions (Deth et al. 2008).

Evidence suggests that Hg disrupts IGF-1 signaling and methionine synthase activity in the brain (Deth et al. 2008). Suzuki and coauthors (2004), for example, found that mRNA expression of the estrogen receptor and insulin-like growth factor 1, which participate in osteoblastic growth and differentiation, was less than the control values after treatment with methylmercury.

Waly and colleagues (2004) found that Thimerosal inhibited both IGF-1- and dopamine-stimulated methylation and eliminated methionine synthase activity in human neuroblastoma cells. The authors stated that their findings outline a novel growth factor signaling pathway that regulates methionine synthase activity and thereby modulates methylation reactions, including DNA methylation. Similarly, Deth and colleagues (2008) found that Thimerosal interferes with PI3-kinase-dependent methionine synthase, resulting in impaired methylation, including DNA methylation that is essential for normal development.

Pilsner and coauthors (2010) found Hg-associated DNA hypomethylation in polar bear brains. They examined 47 polar bears. They found that genomic DNA methylation level was related to postmortem total-brain-Hg levels (an inverse association was seen between these two variables for the entire study population).

Evidence of disruption in IGF-1 signaling, methionine synthase activity, and methylation in autism

Two studies have examined cerebral spinal fluid levels of IGF-1 in autism. First, Vanhala and coauthors (2001) studied whether IGF-I levels might be associated with the development of autism. IGF-I levels were measured in the cerebral spinal fluid (CSF) of 11 children with autism (4 females, 7 males) using a sensitive radioimmunoassay method and compared with levels in 11 control participants (6 females, 5 males). Levels of IGF-I in the CSF were statistically significantly lower in the children with autism than in the control children.

Second, Riikonen and colleagues (2006) measured IGF-1 and -2 from cerebrospinal fluid (CSF) by radio immunoassay in 25 children with autism and in 16 age-matched comparison children without any disability. CSF IGF-1 concentration was significantly lower in patients with autism than in the comparison group. The CSF concentrations of children with autism less than 5 years of age were significantly lower than their age-matched comparisons. The head circumferences correlated with CSF IGF-1 in children with autism but no such correlation was found in the comparison group.

Children with autism also exhibit evidence of impaired methylation (James et al. 2004, 2006, Deth et al. 2008). Deth and coauthors (2008) reviewed the metabolic relationship between oxidative stress and methylation, with particular emphasis on adaptive responses that limit activity of cobalamin and folate-dependent methionine synthase. They stated that children with autism exhibit evidence of oxidative stress and impaired methylation, which may reflect effects of toxic exposure on sulfur metabolism.

Section summary statement

Mercury intoxication can disrupt IGF-1 signaling, methionine synthase activity, and methylation in the brain. The same abnormalities are also found in ASD.

VASCULAR ENDOTHELIAL DYSFUNCTIONS, REDUCED BLOOD FLOW

Evidence for Hg-induced vascular endothelial dysfunctions, reduced vascularity and blood flow in the brain

Endothelial cells (ECs) line the interior surface of blood vessels throughout the entire circulatory system. The thin layer of endothelial cells is called the endothelium. The functional integrity of endothelium is critical for the maintenance of blood flow (Wiggers et al. 2008).

In vitro exposure to Hg induces cytotoxicity in endothelial cells (Kishimoto et al. 1995, Wolf and Baynes 2007, Golpon et al. 2003, Mazeriket al. 2007a,b). Several studies report that Hg causes vascular endothelial dysfunction resulting in pathological changes of the blood vessels (Olczak et al. 2010). As typical of Hg intoxication, there are multiple underlying mechanisms.

First, research shows that mercury activates different types of vascular endothelial cell (EC) phospholipases. Peltz and coauthors (2009), for example, found that Hg ($HgCl_2$, Thimerosal, and methylmercury) significantly activated vascular endothelial cell (EC) phospholipase D (PLD) in bovine pulmonary artery ECs (BPAECs). They stated that the study demonstrated the importance of calcium and calmodulin in the regulation of Hg-induced phospholipase D activation, suggesting mechanisms of mercury vasculotoxicity and Hg-induced cardiovascular diseases for both the inorganic (ionic) and simple alkyl (ethyl and methyl) mercury compounds evaluated.

Likewise, Hagele and colleagues (2007) found that $HgCl_2$ (inorganic form), MeHgCl (environmental form), and Thimerosal (EtHgSAL; pharmaceutical form), induced phospholipase D (PLD) activation**.** All the three different forms of Hg significantly induced the decrease of levels of total cellular thiols. The authors stated that the study revealed that Hg induced the activation of PLD in the vascular ECs wherein cellular thiols and oxidative stress acted as signal mediators for the enzyme activation, and that the results underscore the importance of PLD signaling in Hg-induced endothelial dysfunctions.

Similarly, Mazerik and coauthors (2007b) reported that the vascular toxicity stems from inorganic Hg modulating the activity of the vascular endothelial cell (EC) lipid signaling enzyme phospholipase A(2) [PLA(2)], which is an important player in the EC barrier functions. Mazerik and colleagues (2007b) stated that the results suggest that inorganic Hg-induced PLA(2) activation through the thiol and calcium signaling and the formation of bioactive AA metabolites further demonstrated the association of PLA(2) with the cytotoxicity of inorganic Hg in ECs.

Second, oxidative stress appears to be one of the underlying mechanisms in the destruction of endothelial cells. Evidence suggests that Hg accumulation can affect endothelial function by inhibiting NO synthesis and by increasing ROS and lipid peroxidation (Bautista et al. 2009). The vascular endothelium is highly sensitive to oxidative stress (Wiggers et al. 2008). Furieri and coauthors (2011) and Wiggers and colleagues (2008) analyzed the effects of chronic exposure to low Hg doses on endothelial function, and both concluded that Hg promotes endothelial dysfunction of coronary arteries, as shown by decreased NO bioavailability induced by increased oxidative stress.

Third, Hg also appears to exhibit vascular toxicity by disrupting calcium stores. Gericke and colleagues (1993), for example, found that Thimerosal induced changes of intracellular calcium in human endothelial cells. They examined the effects of the -SH oxidizing agent Thimerosal on the intracellular calcium concentration in single endothelial cells from human umbilical cord vein and found that concentrations higher than 10 μM induced a long lasting increase in intracellular calcium (Thimerosal opens a pathway for Ca^{2+} entry from the extracellular side).

Fourth, Asadi and coauthors (2010) reported that $HgCl_2$ and Thimerosal can stimulate human mast cells to release vascular endothelial growth factor (VEGF), which is also vasoactive and pro-inflammatory. This also contributes to the Hg-induced EC toxicity.

The end result of the Hg-induced EC toxicity is pathological changes in the blood vessels. Olczak and colleagues (2010), for example, examined the effects of early postnatal administration of Thimerosal (4 i.m. injections, 12 or 240 μg THIM-Hg/kg, on postnatal days 7, 9, 11 and 15) on brain pathology in Wistar rats. Numerous neuropathological changes were observed in young adult rats which were treated postnatally with Thimerosal. They included: ischaemic degeneration of neurons and "dark" neurons in the prefrontal and temporal cortex, the hippocampus and the cerebellum, pathological changes of the blood vessels in the temporal cortex, diminished synaptophysin reaction in the hippocampus, atrophy of astroglia in the hippocampus and cerebellum, and positive caspase-3 reaction in Bergmann astroglia.

It is important to note that decreased cerebral blood flow appears to be a result of the Hg-induced EC toxicity. For example, Hargreaves and coauthors (1988) gave MeHgCl to rats in a neurotoxic dose regimen (6 daily doses of 8 mg kg^{-1} p.o.) and also measured regional cerebral blood flow using iodoantipyrine. The authors found that blood flow was reduced throughout the brain.

The tragedy in Minamata has also provided evidence for decrease blood flow in the brain of humans as a result of Hg exposure. At least two studies have found that the victims of Minamata had decreased brain blood flow. Itoh and coauthors (2001) found decreased cerebellar blood flow in patients with Minamata disease. Even in patients without cerebellar atrophy, blood flow was significantly decreased. This finding was corroborated later by Taber and Hurley (2008).

Evidence for vascular endothelial dysfunctions, reduced vascularity and brain blood flow in autism

Several studies have shown abnormal platelet reactivity and vascular endothelium activation in children with autism (Yao et al. 2006), as well as decreased cerebral blood flow in the brain (Galuska et al. 2002). Yao and colleagues (2006), for example, evaluated the vascular phenotype in children with autism by measuring urinary levels of isoprostane F(2alpha)-VI, a marker of lipid peroxidation; 2,3-dinor-thromboxane B(2), which reflects platelet activation; and 6-keto-prostaglandin F(1alpha), a marker of endothelium activation. These were measured by means of gas chromatography-mass spectrometry in subjects with autism and in healthy control subjects. The results showed that, compared to the levels in the control subjects, children with autism had significantly higher urinary levels of isoprostane $F_{2\alpha}$-VI, $_{2,3}$-dinor-thromboxane B_2, and 6-keto-prostaglandin $F_{1\alpha}$. Lipid peroxidation levels directly correlated with both vascular biomarker ratios. The authors stated that the results indicate enhanced oxidative stress, platelet and vascular endothelium activation.

Many studies report decreased cerebral blood flow (rCBF) (or hypoperfusion) in autism (Zilbovicius et al. 1995, 2000, Wilcox et al. 2002, Ito et al. 2005, Degirmenci et al. 2008). These studies used positron emission tomography (PET) and single-photon emission computed tomography (SPECT) as a means of examining regional cerebral blood in autism as compared to controls. All the studies consistently found hypoperfusion in autism as compared to the controls. Examples are as follows:

Using SPECT, Wilcox and coauthors (2002), for example, examined blood flow in 14 individuals with ASD and 14 controls ranging in age from 3 to 37 years and found significant hypoperfusion in the prefrontal areas of individuals with ASD as compared to controls in every case. Other areas of the brain reported to have decreased blood flow in autism using SPECT include: (1) prefrontal cortex, medial frontal cortex, anterior cingulate cortex, medial parietal cortex, and/or anterior temporal cortex (Sasaki et al. 2010); (2) frontal cortex (Zilbovicius et al. 1995); and (3) bilateral insula, superior temporal gyri and left prefrontal cortices (Ohnishi et al. 2000). Ohnishi and colleagues (2000) also reported that analysis of the correlations between syndrome scores and regional cerebral blood flow (rCBF) revealed that each syndrome was associated with a specific pattern of perfusion in the limbic system and the medial prefrontal cortex. They stated that the perfusion abnormalities seem to be related to the cognitive dysfunction observed in autism, such as abnormal responses to sensory stimuli, and the obsessive desire for sameness.

Examining cerebral perfusion abnormalities in children with autism in a segmental quantitative SPECT study, Gupta and Ratnam (2009) found generalized hypoperfusion in all of the cases (n=10) as compared to controls**.** The authors stated that frontal and prefrontal regions revealed maximum hypoperfusion and that subcortical areas also indicated hypoperfusion.

Galuska and coauthors (2002) reported decreased blood flow with left-sided dominance was found bifrontally and bitemporally in the autism (perfusion-metabolism mismatch). Even in those with high functioning autism, hypoperfusion is reported. Ito and colleagues (2005), for example, examined regional cerebral blood flow pattern in subjects with high-functioning autism and found hypoperfusion.

Using PET, areas that have been reported to show hypoperfusion in ASD as compared to controls include: (1) both temporal lobes centered in associative auditory and adjacent multimodal cortex (Zilbovicius et al. 2000); and (2) temporal lobe (Brunelle et al. 2009). No studies were found that reported normal perfusion in the brain in autism.

Section summary statement

Mercury intoxication can result in vascular dysfunction and decreased blood flow in various areas of the brain. These symptoms are also commonly found in those with ASD.

AMYLOID PRECURSOR PROTEIN

Evidence of Hg-induced increase in the expression of the amyloid precursor protein

Several studies show that Hg induces an increase in the expression of the amyloid precursor protein and the formation of insoluble beta-amyloid (Mutter et al. 2004, Haley 2005, Monnet-Tschudi et al. 2006, Lozac 2010). Using neuroblastoma cells, Olivieri and coauthors (2000), for example, examined the potential

pathophysiological mechanisms of inorganic Hg ($HgCl_2$) on oxidative stress, cell cytotoxicity, beta-amyloid production, and tau phosphorylation. The authors found that exposure of cells to 50 µg/L (180 nM) $HgCl_2$ for 30 min induces a 30% reduction in cellular GSH levels and the release of beta-amyloid peptide. Olivieri and colleagues (2002) examined the role of estrogen (beta-estradiol) neuroblastoma cells which had been exposed to Hg and found that Hg induced oxidative stress and cell cytotoxicity and increased the secretion of beta-r 1-40 and 1-42. These negative effects were blocked by the pretreatment of cells with beta-estradiol. Importantly, Hock and colleagues (1998) found that levels of amyloid beta-peptide in cerebral spinal fluid correlated with Hg blood levels in adults with Alzheimer's disease.

Evidence of an increase in the amyloid precursor protein in autism

Although the studies that have examined this issue are limited, there is evidence to show excess amyloid precursor protein in autism. Sokol and coauthors (2006) examined acetylcholinesterase, plasma neuronal proteins, secreted beta-amyloid precursor protein (APP), and amyloid-beta 40 and amyloid-beta 42 peptides in children with and without autism. Children with severe autism and aggression expressed secreted beta-amyloid precursor protein at two or more times the levels of children without autism and up to four times more than children with mild autism. In addition, Ray and colleagues (2011) examined plasma samples of amyloid-β (Aβ) precursor protein-alpha form (sAPPα), sAPPβ (beta form), Aβ peptides, and brain-derived neurotrophic factor (BDNF) in 18 control, 6 mild-to-moderate, and 15 severely autistic participants. They found that sAPPα levels are increased and BDNF levels decreased in the plasma of patients with severe autism as compared to controls. The authors stated that their study provides evidence that sAPPα levels are generally elevated in severe autism.

Section summary statement

Mercury intoxication can result in an increase in the presence of amyloid precursor protein in the brain and evidence suggests this is also the case in autism.

GRANULE AND PURKINJE CELL DEGENERATION

Evidence of granule and Purkinje cell degeneration and loss in the cerebellum from mercury

Numerous studies have shown that mercury damages granule and Purkinje cells in the cerebellum, with effects that include: (1) heterotopic location (altered location; Sakamoto et al. 2002, Carvalho et al. 2008); (2) degenerative changes (Cinca et al. 1980, Bertossi et al. 2004, Olczak et al. 2010); (3) mercury accumulation (Magos et al. 1985, Warfvinge 2000); and (4) significant cell loss (Hua et al. 1995, Sorensen et al. 2000, Youn et al. 2002). Granule and Purkinje cell degeneration and cell loss has been found to result from: (1) metallic mercury vapor (Hua et al. 1995, Sorensen et al. 2000); (2) methylmercury (Warfvinge 2000, Sakamoto et al. 2002, Eto 2006, Carvalho et al. 2008); (3) mercuric sulfide (HgS; Youn et al. 2002); (4) ethylmercury (Cinca et al. 1980) and specifically Thimerosal (Hornig et al. 2004). Also noted, is Purkinje cell axonal retraction bulbs from methylmercury exposure (Hunter and Russell 1954). Importantly, Warfvinge (2000) found evidence that mercury is trapped in the cerebellum over a long period of time. In a study which replicated Minamata disease using the common marmoset, Purkinje and granule cells in the test subjects were found to be decreased in number in the cerebellum, depending on the estimated dose of the methylmercury compound and the duration of the exposure (Eto 2006).

Evidence of granule and Purkinje cell degeneration and loss in the cerebellum in autism

One of the most consistent neurological abnormalities found in persons with autism is marked Purkinje cell loss in the cerebellum (as determined by histopathological postmortem examination) and atrophy of the cerebellar folia (as determined by *in vivo* neuroimaging) (Ritvo et al. 1986, Courchesne 1991, 1995, Kemper and Bauman 1993, Bailey et al. 1998, Lee et al. 2002, Palmen et al. 2004, Whitney et al. 2008, 2009). According to Ritvo and coauthors (1986), for example, the Purkinje cells in the vermis of the cerebellum were approximately 15 standard deviations below the mean, and approximately 8 standard deviations below the mean bilaterally in the cerebellar hemispheres in the subjects with autism, as compared to

normal controls. Reduction in granule cell number has also been noted by histopathological post-mortem examination in autism (Bauman and Kemper 1984, 1985, 1994).

Section summary statement

Mercury intoxication can result in loss of granule and Purkinje cells in the cerebellum, which is a consistent postmortem histopathological abnormaility found in the brains of those with ASD.

PRO-INFLAMMATORY CYTOKINES

Evidence of mercury induced increased pro-inflammatory cytokine levels in the brain (TNF-α, IFN-γ, IL-1beta, IL-6, IL-8)

Several studies show that mercury exposure increases cytokine levels peripherally (Noda et al. 2003). For example, mercury-exposed gold miners have significantly higher concentrations of pro-inflammatory cytokines interleukin-1beta (IL-1beta), tumor necrosis factor-alpha (TNF-alpha or TNF-α), and interferon-gamma (IFN-gamma or IFN-γ) in serum as compared to the diamond and emerald miners (Gardner et al. 2010). Mercury based dental amalgams are found to increase production of TNF-α and IFN-γ cytokines (Podzimek et al. 2010). Nyland and coauthors (2012) found that low dose inorganic mercury induced macrophage infiltration and mixed cytokine response in the heart during acute myocarditis, including significantly increased interleukin-12, IL-17, interferon-γ, and TNF-α levels.

In mice, Kim and colleagues (2003) found that oral exposure to inorganic mercury altered T lymphocyte phenotypes and cytokine expression. They stated that mercury altered the expression of inflammatory cytokines (TNF-α, IFN-γ, and IL-12), c-myc, and major histocompatibility complex II, in various organs.

Importantly, this same effect is found in the mercury exposed brain. For example, in a recent study by Curtis and coauthors (2010), chronic mercury exposure (10 week mercury exposure of 60 ppm $HgCl_2$ in drinking water) in the prairie vole resulted in a male-specific increase in TNF-α protein expression in the cerebellum and hippocampus. Importantly, the cerebellum and hippocampus are two of the main areas found to be affected in ASD (Kern and Jones 2006). As mentioned in the section on microglia, Eskes and coauthors (2002) found in brain cell cultures that isolated microglia were directly activated by noncytotoxic MeHgCl treatment and there was also an increase local IL-6 release. They stated that microglial cells are directly activated by MeHgCl and that the interaction between activated microglia and astrocytes can then increase local IL-6 release.

Evidence of increased pro-inflammatory cytokine levels in the brain in autism (TNF-α, IFN-γ, IL-1β, IL-6, IL-8)

Imbalances in the regulation of pro-inflammatory cytokines have been increasingly correlated in ASD (Lee et al. 2010) and, in particular, cytokine alteration of TNF-α is increased in autistic populations (Cohly and Panja 2005). A number of studies have shown that TNFα, IFNγ, IL-1β, and IL-12 were increased in the peripheral blood of ASD patients (Zimmerman et al. 2005, Molloy et al. 2006, Ashwood and Wakefield 2006).

Similarly, studies show elevated immune response in the brains and spinal cords of autistic patients. Li and colleagues (2009), for example, showed that proinflammatory cytokines (TNF-α, IL-6 and GM-CSF), Th1 cytokine (IFN-γ) and chemokine (IL-8) were significantly increased in the brains of ASD patients compared with the controls. A study by Vargas and coauthors (2005) demonstrated that tumor growth factor (TGF)-β1, derived from neuroglia, was significantly increased in the middle frontal gyrus (MFG) of autistic patients, while macrophage chemoattractant protein (MCP)–1, IL-6 and IL-10 were increased in the anterior cingulate gyrus (ACG). In addition, using protein array approach, Vargas and colleagues (2005) also found that MCP-1, IL-6, IL-8 and IFN-γ were significantly increased in the cerebrospinal fluid (CSF). TNFα was also shown to be increased in the cerebral spinal fluid of autistic patients by Chez and coauthors (2007). Chez and colleagues (2007) stated that the elevation of cerebrospinal fluid levels of TNF-α was significantly higher (mean = 104.10 pg/mL) than concurrent serum levels (mean = 2.78 pg/mL) in all of the patients studied. They stated that the ratio was significantly higher than the elevations reported for other pathological states for which cerebrospinal fluid and serum TNF-α levels have been simultaneously measured and that this finding may provide insight into central nervous system inflammatory mechanisms in autism.

According to Wei and colleagues (2012), a number of studies showed that cytokines are increased in the blood, brain, and cerebrospinal fluid of autistic sub-

jects and that elevated IL-6 in the autistic brain has been a consistent finding. Importantly, Wei and coauthors (2011, 2012) completed two studies that showed that IL-6 may be involved in autism. First, in 2011, they showed that IL-6 was significantly increased in the cerebellum of autistic subjects as compared to age-matched controls and they found that IL-6 over-expression in granule cells caused impairment in their adhesion and migration. Then in 2012, Wei and colleagues found that that mice with elevated levels of IL-6 in the brain display many autistic features, including impaired cognitive abilities, deficits in learning, abnormal anxiety traits and habituation, as well as decreased social interactions. In addition, IL-6 elevation caused alterations in excitatory and inhibitory synaptic formations and disrupted the balance of excitatory/inhibitory synaptic transmissions and also resulted in an abnormal shape, length and distributing pattern of dendritic spines.

Section summary statement

Mercury intoxication can result in elevated proinflammatory cytokines, specifically, TNF-α, IFN-γ, IL-1beta, IL-6, and IL-8, which are also elevated in the brains of those diagnosed with ASD. Increased level of these cytokines is postulated as one of the mechanisms leading to histopathological changes found in autism.

ABERRANT NUCLEAR FACTOR KAPPA-LIGHT-CHAIN-ENHANCER OF ACTIVATED B CELLS (NF-KB)

Evidence of mercury induced aberrant nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)

Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) is a protein found in almost all cell types mediating regulation of immune response by induction of expression of the inflammatory cytokines and chemokines and establishing a feedback mechanism that can produce chronic or excessive inflammation (Young et al. 2011). NF-κB activation induces numerous proinflammatory gene products including cytokines, cyclooxygenase-2 (COX-2), and inducible nitric oxide synthase (iNOS) (Park and Youn 2011).

Several studies show that mercury exposure results in aberrant activation of NF-κB (Valko et al. 2005, Assefa et al. 2011, Pal et al. 2011). For example, Park and Youn (2011) found that mercury activates NF-κB, resulting in the induced expression of COX-2 and iNOS. They stated that the results suggest that mercury can induce inflammatory diseases by lowering host defense. Korashy and El-Kadi (2008) found that Hg^{2+} causes the induction of oxidative stress markers, such as ROS and heme oxygenase-1 and the depletion of cellular glutathione content, associated with NF-κB and AP-1 activation.

Dong and coauthors (2001) examined the effects of methylmercury chloride on DNA binding activities of NF-κB in developing rat cerebella and cerebra using electrophoretic mobility shift assays(EMSAs). They found that NF-κB I and NF-κB II DNA binding activities of nuclear protein extracts from rat cerebra exposed to methylmercury chloride in uterus were lower than in control groups on postnatal day 3 and 7, while that from rat cerebella was higher than control groups. They also found that the greater the level of methylmercury chloride, the higher the NF-κB DNA binding activities of nuclear protein extracts.

Evidence of aberrant nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) in the brains of autistic patients

The neuroinflammation in autism appears to be strong and chronic. Recent research suggests that this exaggerated response may be due to aberrant NF-κB expression in autism, which can produce chronic and/or excessive inflammation (Young et al. 2011). A recent study by Young and colleagues (2011) examined NF-κB in human postmortem samples of orbitofrontal cortex tissue of autistic patients as compared to controls. According to the authors, neurons, astrocytes, and microglia all demonstrated increased extranuclear and nuclear translocated NF-κB p65 expression in brain tissue from ASD donors relative to samples from matched controls. These between-groups differences were increased in astrocytes and microglia relative to neurons, but particularly pronounced for highly mature microglia. Measurement of pH in homogenized samples demonstrated a 0.98-unit difference in means and a strong (F=98.3; P=0.00018) linear relationship to the expression of nuclear translocated NF-κB in mature microglia. Young and col-

leagues (2011) summarized that NF-κB is aberrantly expressed in orbitofrontal cortex in patients with ASD, as part of a putative molecular cascade leading to inflammation, especially of resident immune cells in brain regions associated with the behavioral and clinical symptoms of ASD. Their study provides further evidence of neuroinflammation that may be categorized as excessive in ASD.

Naik and coauthors (2011) examined NF-κB in peripheral blood samples of 67 children with autism and 29 control children using electrophoretic mobility shift assay (EMSA). They stated that there was a significant increase in NF-κB DNA binding activity in peripheral blood samples of children with autism and when the fold increase of NF-κB in autism cases (n=67) was compared with that of controls (n=29), there was a significant difference (3.14 vs. 1.40, respectively; P<0.02). They concluded that autism may arise, at least in part, from an NF-κB pathway gone awry.

Evidence suggests that the equivalent of a vicious cycle can occur where microglia produce oxidative products and then increased intracellular ROS, in turn, activates a redox-sensitive nuclear factor-κB (NF-κB) to provoke excessive neuroinflammation. According to Nakanishi and coauthors (2011), this can result in memory deficits and the prolonged behavioral consequences.

Section summary statement

Mercury intoxication can induce NF-κB activation which may produce chronic or excessive inflammation in the brain. Such changes are also found in the brains of those with ASD.

DISCUSSION

Sources and levels of mercury

Mercury accumulates in both the body and environment, and evidence indicates that background mercury levels are increasing. Environmental studies and human studies warn of increasing mercury levels. According to the United States Department of Interior/US Geological Survey (2002), the US has a growing mercury level problem. The problem is not confined to the United States. A 2009 study of mercury levels in the oceans led by scientists from Harvard University and the U.S. Geological Survey, found that the ocean's mercury levels have risen about 30% over the last 20 years, predominately from industrial emissions (Sunderland et al. 2009). The authors stated that mercury is circulated over vast distances and that Asia's burning of coal is the primary source of mercury emissions worldwide.

These increased levels of mercury in the environment are reflected in human studies. Laks (2009), for example, recently reported on time trends of blood inorganic mercury (I-Hg) levels in 6 174 women of ages 18–49, in the National Health and Nutrition Examination Survey (NHANES) 1999–2006 data sets. Laks found that in the US population, proportion of I-Hg detection rose sharply from 2% in 1999–2000 to 30% in 2005–2006. In addition, the population averaged mean I-Hg concentration rose significantly over that same period from 0.33 to 0.39 μ/L. I-Hg was significantly associated with age suggesting bio-accumulation. Laks (2009) stated that the study provided evidence that I-Hg deposition within the human body is a cumulative process, increasing with age and in the population over time, since 1999, as a result of chronic mercury exposure.

If blood mercury levels in women are increasing, as suggested by the NHANES data, then it is safe to assume that fetal exposure to mercury is also increasing. Studies consistently show that fetal cord blood mercury levels are higher than the maternal blood levels (Nyland et al. 2011, Lederman et al. 2008). For example, Nyland and coauthors (2011) found that fetal cord blood mercury levels were higher (1.35 times) than in their mother's blood. The mercury levels in mother and child were highly correlated (correlation coefficient of r=0.71; 95%). They also found that the maternal and fetal cord blood mercury levels were positively correlated with inflammatory cytokines (IL-1β, IL-6, and TNF-α). Lederman and colleagues (2008) examined levels of total mercury in cord and maternal blood in US women and found that the total mercury level in the fetal cord blood was over twice the levels in the blood of the mothers. The study showed that 1 in 3 US infants has a cord blood total mercury level above the Environmental Protection Agency (EPA) limit that is considered safe, whereas one in six mothers of these same children had blood

mercury levels above the EPA limit. After controlling for fish/seafood consumption and other confounders, long-term follow-up revealed that the logarithm of the cord blood mercury was inversely associated with the Bayley Scales of Infant Development psychomotor score at 36 months, and with the Performance, Verbal, and Full IQ scores on the Wechsler Preschool and Primary Scale of Intelligence, Revised (WPPSI-R), at 48 months. Importantly, the maternal blood levels for total blood mercury in the Lederman and other (2008) study (where developmental consequences in the children were noted) were typical of the levels in the NHANES data representative of women across the country. Furthermore, the maternal blood mercury level was within the EPAs safety limit for mercury. Several studies besides the Lederman and coauthors (2008) study have also shown that even though the mother's blood level was within the level considered to be safe by the EPA, there were still associated decrements in cognitive function in the children (Jedrychowski et al. 2006, Lederman et al. 2008, Björnberg et al. 2003).

Significantly, the US has seen a dramatic increase in the rates of neurodevelopmental disorders in children in general. Neurodevelopmental disabilities in the US have so dramatically increased in the last decade, that at a current rate of 1 in 6 children, neurodevelopmental disorders are now considered common (Boyle et al. 2011).

The relationship between mercury body-burden and exposure in children appears to be measurable. For example, a study by Kern and colleagues (2011b) examined urinary porphyrin levels (a biomarker of Hg body burden) in American children (in Texas) as compared to French children (in eastern France). These two groups were chosen because of mercury exposure differences based on two factors: (1) exposure from Thimerosal in vaccines and (2) mercury from coal burning plants. In comparing the relative presence of Thimerosal in vaccines administered in the United States (US) and France, Hessel (2003) reported that in 1999 in the US, Thimerosal was present in approximately 30 different childhood vaccines, whereas there were only 2 in France. In the US, Thimerosal is still in the influenza vaccines recommended for pregnant women, infants, and children to be given every year and it is also in the H1N1 vaccine (both of which are reportedly seldom administered in France). In regard to coal burning, energy produced from coal in France in 2003 was 6% *versus* 51% in the US and 36.5% in Texas (Encyclopedia of the Nations 2007). Plus, the children in the Texas group lived in an area that has one plant alone producing about 1 500 lbs of mercury per year which, because of the prevailing winds, is dispersed over the area in which they reside. The study found that the US children have a significantly increased body-burden of mercury in comparison to the body-burden of mercury in the matched French children.

Some sources of Hg exposure include industrial emissions, Thimerosal in vaccines, high fructose corn syrup, compact fluorescent light bulbs, fish, dental amalgam fillings and dental waste, and emissions from crematories. Several of these sources have been found to be associated with autism. Scientists have found a relationship between autism and (1) Hg emissions from coal burning plants (Palmer et al. 2006, 2009, Windham et al. 2006), (2) Thimerosal in vaccines (Geier et al. 2008, Young et al. 2008, Gallagher and Goodman 2010), and (3) dental amalgam fillings (Geier et al. 2009d). In addition, anecdotal reports have suggested a higher rate of autism near plants that manufacture compact fluorescent light bulbs.

How the parallels described in this review add to the evidence that has already been compiled in the continuing examination of mercury and autism

This current review of the parallels between Hg intoxication and the brain pathology in ASD reveals many parallels. Even though associations are not proof of causality, these parallels add to the evidence that is already compiled in ASD. Furthermore, these parallels argue against theories questioning the role of mercury in the pathology of autism on the basis that these pathologies are dissimilar. On the contrary, these parallels show that the pathology of the brain in ASD and mercury intoxication is quite similar.

Other metals, toxins, and brain insults and ASD pathology

It, it is important to note, however, that other heavy metals, toxins and insults can cause patho-

logical changes similar to those caused by Hg which should also be taken into consideration. Particularly lead (Pb) can cause pathological changes similar to pathology caused by Hg (Korashy and El-Kadi 2008). However, evidence suggests that mercury is in a class of its own in that it can cause all of the types of pathology seen in autism. For example, the authors could find no evidence that exposure to Pb decreases cerebral blood flow which is a common finding in autism.

Most of the studies that have examined the relationship between autism severity and Hg have found that there is a positive correlation between Hg body burden and autism symptom severity. This includes studies that have examined: (1) urinary mercury levels (Adams et al. 2009), (2) urinary precoproporphyrins levels, a biomarker of Hg toxicity (Nataf et al. 2006, Geier and Geier 2007, Geier et al. 2009b, Kern et al. 2010), (3) hair mercury levels (Holmes et al. 2003, Elsheshtawy et al. 2011, Lakshmi Priya and Geetha 2011), and (4) nail mercury levels in ASD (Lakshmi Priya and Geetha 2011). Furthermore, direct measures of total mercury levels in cerebellar samples from the brains of deceased subjects with autism positively correlated with the level of oxidative stress markers found in the brain tissue (Sajdel-Sulkowska et al. 2008).

Although most of the research shows a relationship between ASD and Hg, some do find a relationship with both Pb and Hg. For example, Lakshmi Priya and Geetha (2011) found a significant elevation ($P<0.001$) in the levels of toxic metals Pb and Hg in both hair and nail samples of a group of autistic children when compared to the control group. The elevation was more pronounced in the more severely affected children when compared to children with moderate to mild symptom severity. Elsheshtawy and collegues (2011) had similar findings. They found highly significant differences between the level of Pb, Hg, and copper in the hair of children with autism compared with controls, and there was a positive correlation with autism severity (using the Childhood Autism Rating Scale) with both Hg and copper, but not Pb (however, the intelligence quotient had significant negative correlation with the level of Pb in the hair). In addition, Adams and coauthors (2009) found a positive relationship between urinary Pb and Hg levels (after chelation) and ASD severity.

To the authors' knowledge, no studies have found elevated Pb levels in ASD without finding concomitant elevated Hg levels. Taken together these findings suggest synergistic effects, which is the topic of the following section.

Mercury and synergism: its relevance in autism

Synergism (the interaction of discrete elements or agents such that the total effect is greater than the sum of the individual effects) is frequently reported in mercury research. Mercury has been found to work synergistically with other metals, toxins, and pathogens (Mutter 2011). For example, mercury has been found to have synergistic effects with the following substances: (1) polychlorinated biphenyls (PCBs; Bemis and Seegal 1999, Roegge et al. 2004, 2006); (2) dithiothreitol (Hultberg et al. 2001); acetaminophen (Zwiener et al. 1994); (3) flame retardant PBDE 99 (2,2',4,4',5-pentabromodiphenyl ether; Fischer et al. 2008); (4) cadmium (Mohan et al. 1986, Yu et al. 2008); (5) lead and manganese (Fernández and Beiras 2001, Papp et al. 2006); (6) endotoxin (a product released by the cell walls of gram negative bacteria; Rumbeiha et al. 2000); (7) ethanol (Turner et al. 1981); and (8) testosterone (Geier et al. 2010).

Importantly, mercury can also increase susceptibility of affected individuals to bacterial and viral infections (Shen et al. 2001). Ilbäck and coauthors (1996), for example, found that viral infections in mice were made worse in the presence of mercury (MeHg). Christensen and colleagues (1996) examined herpes simplex virus type 2 (HSV-2) in mice and found that $HgCl_2$ "aggravated" the infection. Ellermann-Eriksen and coauthors (1994) examined the influence of $HgCl_2$ on resistance to generalized infection with herpes simplex virus type 2 (HSV-2) in mice. They found that mercury, by interfering with the early macrophage-production of cytokines, disabled the early control of virus replication**,** leading to an enhanced infection. Bennett and coauthors (2001) found that concentrations of Hg were significantly higher in porpoises that died of infectious disease compared to healthy porpoises that died from physical trauma.

Furthermore, not only does mercury increase susceptibility of affected individuals to bacterial and viral infections, several studies show that viral

infections cause an increase in brain mercury levels (Ilbäck et al. 2005, 2007, Frisk et al. 2008). Ilbäck and coauthors, in 2005, found that coxsackie virus B3 infection in mice triggered a twofold increase in the concentration of Hg in the brain. Later, Ilbäck and colleagues (2007), again, found that viral infection brought about a significant increase in Hg in the brain. Moreover, the increase in the brain was positively correlated to a concomitant decrease ($P<0.05$) of Hg in serum. Likewise, Frisk and coauthors (2008) found that a common viral infection in mice increased the amount of Hg in the brain by 52% compared to controls.

In addition, the presence of Hg in the brain can potentially activate the brain's immune or neuroinflammatory response to a peripheral or central immune trigger. [It is important to note that the brain's immune system can be activated by brain infection and inflammation, as well as, systemic infection and inflammation (Teeling and Perry 2009).] Potentiation of the brain's immune response can result because Hg: (1) lowers GSH levels (which can cause microglia mediated neurotoxicity; Lee et al. 2010) and (2) alters NF-κB functioning (which can produce chronic or excessive neuroinflammation; Young et al. 2011). Thus, once the brain's immune system is triggered, Hg can then potentiate the type of neurodegeneration that results from sustained immune activation in the brain by promoting an exaggerated, sustained response (Banks and Kastin 1991, Banks et al. 1995). As mentioned earlier, sustained neuroinflammation can result in neuronal damage and loss of synaptic connections (Gehrmann et al. 1995). This issue of mercury working together with the brain's immune system to bring about acute symptoms and regression following vaccines has been theorized to explain the connection between Thimerosal-preserved-vaccines and the risk of autism.

Research evidence suggests that other metals, toxins, and pathogens may be involved in the underlying pathology in autism (Rose et al. 2008). For example, several studies have shown that toxic metal levels of arsenic, cadmium, and lead are also different in children with autism as compared to typically developing children (Kern et al. 2007). In addition, it has been suggested from previous studies that viral infections may play a role in the pathology (Cohly andPanja 2005, Libbey et al. 2005, Fatemi et al. 2008). Atladóttir and coauthors (2010), for example, investigated the association between hospitalization for infection in the perinatal/neonatal period or childhood and the diagnosis of ASD in 1 418 152 children (of which 7 379 children were diagnosed as having ASD). Children admitted to the hospital for any infectious disease displayed an increased rate of ASD diagnosis.

These examples of synergism suggest that mercury can potentiate or work concomitantly with other possible contributors in the underlying pathology in ASD. Rose and colleagues (2008) who found a genetic susceptibility to mercury and lead in children with autism stated that the individual risk of developmental neurotoxicity with exposure to environmentally relevant levels of lead and mercury is likely to be determined by genetic susceptibility factors as well as additive interactions with other environmental pollutants, cumulative dose, and the developmental stage of exposure.

CONCLUSION

In 2000, Bernard and coauthors published a review of the similarities between Hg poisoning and the symptoms of autism. In their review, Bernard and colleagues pointed out 79 similarities between the symptoms of autism and the symptoms of mercury intoxication. Since that review, many more studies have become available to allow for the current comparison of the specific effects of Hg exposure in the brain and the similar pathological findings in those diagnosed with ASD (see Table I for a summary of the parallels between symptoms of Hg intoxication in the brain and the brain pathology in ASD). The combined similarities outlined by these two reviews are too numerous to be a result of chance. Although there may be genetic or developmental components to autism, the evidence in this current review of the brain findings in autism clearly indicates the reality of brain injury in ASD; moreover, the brain injury symptoms which characterize autism closely correspond to those seen in sub-acute Hg intoxication. The evidence presented in this paper is consistent with mercury being identified as either causal or contributory, working synergistically with other compounds or pathogens in producing the brain pathology observed in those diagnosed with ASD.

Table I

Parallels between mercury intoxication in the brain and the brain pathology in ASD	
Hg Effects on the CNS	Brain Pathology in Autism
Large, long-range axon degeneration	Loss of large, loss range axons Long-range underconnectivity
Dentritic overgrowth	Overgrowth Short-range over connectivity
Neuroinflammation Microglial/astrocytic activation Brain immune response activation	Neuroinflammation Microglial/astrocytic activation Brain immune response activation
Elevated GFAP	Elevated GFAP
Oxidative stress and lipid peroxidation	Oxidative stress and lipid peroxidation
Dose-dependent relationship between mercury levels and oxidative stress	Dose-dependent relationship between mercury levels and oxidative stress
Decreased reduced glutathione levels and elevated oxidized glutathione	Decreased reduced glutathione levels and elevated oxidized glutathione
Mitochondrial dysfunction	Mitochondrial dysfunction
Disrupts calcium homeostasis and signaling	Disruption in calcium homeostasis and signaling
Inhibits glutamic acid decarboxylase activity	Decreased brain levels of glutamic acid decarboxylase and GABA
Disrupts GABAergic and glutamatergic homeostasis	Disrupted GABAergic and glutamatergic homeostasis
Inhibits IGF-1 and methionine synthase activity	Altered IGF-1 levels and methionine synthase activity
Impairs methylation	Impaired methylation
Vascular endothelial cell dysfunction and pathological changes of the blood vessels	Vascular endothelial cell dysfunction and pathological changes of the blood vessels
Decreased cerebral/cerebellar blood flow	Decreased cerebral/cerebellar blood flow
Increase in the amyloid precursor protein	Increase in the amyloid precursor protein
Granule and Purkinje neuron loss in the cerebellum	Granule and Purkinje neuron loss in the cerebellum
Increases in pro-inflammatory cytokine levels (TNF-α, IFN-γ, IL-1β, IL-6, IL-8)	Increased pro-inflammatory cytokine levels (TNF-α, IFN-γ, IL-1β, IL-6, IL-8)
Aberrant nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)	Aberrant nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB)

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