

# Aluminum Induced Immunoexcitotoxicity in Neurodevelopmental and Neurodegenerative Disorders

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**Abstract:** A great deal has been learned about the neurotoxicity of aluminum over the past two decades in terms of its ability to disrupt cellular function. Newer evidence suggests that a more central pathophysiological mechanism may be responsible for much of the toxicity of aluminum and aluminofluoride compounds on the brain. This mechanism involves activation of the brain's innate immune system, primarily the microglia, with a release of neurotoxic concentrations of excitotoxins and pro-inflammatory cytokines, chemokines and immune mediators. A large number of studies suggest that excitotoxicity plays a significant role in the neurotoxic action of a number of metals, including aluminum. Recently, researchers have found that while most of the chronic neurodegenerative effects of these metals are secondary to prolonged inflammation, it is the enhancement of excitotoxicity by the immune mediators that is responsible for most of the metal's toxicity. This enhancement occurs *via* a crosstalk between cytokine receptors and glutamate receptors. The author coined the name immunoexcitotoxicity to describe this process. This paper reviews the evidence linking immunoexcitotoxicity to aluminum's neurotoxic effects.

**Keywords:** Aluminum, aluminofluoride complex, excitotoxicity, immunoexcitotoxicity, microglial activation, nanoscaled aluminum, neurodegeneration, sickness behavior.

## 1. INTRODUCTION

Aluminum is the third most abundant metal on earth and compelling evidence suggest that it is a major neurotoxin and disrupter of neurological function. It has also been established that aluminum is not essential to human metabolism at any concentration [1]. Humans are exposed to aluminum by a number of routes, including foods, industrial exposures, drinking water, pharmaceuticals and vaccines. While absorption from the gut is quite poor, that introduced *via* parenteral fluids and vaccines is completely absorbed and distributed throughout the body [2]. Interestingly, there appears to be a common mechanism by which aluminum and other known neurotoxic metals (i.e., manganese, mercury and lead) trigger and propagate their toxic actions within the central nervous system (CNS). This mechanism is immunoexcitotoxicity. This term was coined by the author to link previously described interactions between the immune system and the excitotoxic mechanism [3]. The purpose of this review is to discuss the evidence linking immunoexcitotoxicity to aluminum's neurotoxic effects.

## 2. THE BASIC PRINCIPLES OF IMMUNOEXCITOTOXICITY

The histological basis for immunoexcitotoxicity is the brain's innate immune system, primarily involving microglia and astrocytes. Microglia make up 5 to 15% of the cells in the central nervous system (CNS) cortical grey matter, hippocampus, olfactory telencephalon and basal ganglion [4]. Under normal conditions, the brain's microglia exist in what has been referred to as a resting or ramified state, even

though these cells are far from resting [5-7]. In this mode, microglia are constantly extending and retracting pseudopodia, sampling the surrounding microenvironment to assure homeostatic conditions are maintained. In this ramified state they can secrete basal levels of neurotrophic substances to maintain connectivity and the integrity of synapses and dendrites and actively remove excess glutamate from the extracellular environment.

Microglia can activate rapidly in response to various disturbances [8] Once activated, microglia can either assume a reparative/beneficial phenotype (and secrete a number of anti-inflammatory and trophic factors essential for neuronal survival), or a predominantly neurodestructive phenotype. The latter is characterized by secretion of pro-inflammatory cytokines, cytotoxic factors and excitotoxins [6-8]. In neurodegenerative diseases such as Alzheimer's (AD), Parkinson's (PD), Huntington's, Pick's, HIV dementia, multiple sclerosis (MS) and amyotrophic lateral sclerosis (ALS), activated microglia are present in large numbers, a condition termed microgliosis, strongly implicating these cells in disease pathology [6]. Currently, the exact conditions determining whether microglial activation is detrimental or beneficial to neuronal survival are not clear [5,6].

The surface of the microglia contain a number of receptors, including receptors for most of the neurotransmitters, pro- and anti-inflammatory cytokines, chemokines, interferons and major histocompatibility complex (MHC) class I and II receptors [6]. Microglia also contain characteristic receptors called pattern recognition receptors (PRR), which are constitutively expressed to identify and bind various pathogen-associated molecular pattern sites (PAMPS) and other non-self molecules [6]. In addition, microglia also express toll-like receptors (TLRs), with TLR 1-9 of the 12 known TLRs being found on microglia membranes [9]. TLRs not

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only recognize microbial antigens but also regulate the magnitude and duration of the immune response. Recognition of various ligands by the PRR can initiate the generation and release of superoxide, *via* activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase [10]. Due to the expression of these numerous immune markers, microglia are often referred to as the nervous system's resident immune cells [5,8].

When considering immunoexcitotoxicity one is concerned with two events regulated by activated microglia: (i) pro-inflammatory cytokine/chemokine release; and (ii) release of excitatory amino acids, particularly glutamate. It is the excitotoxicity component rather than inflammation alone that appears to be the main pathological mechanism for actual damage to neurons and their processes. For example, research shows that even exposure to high concentrations of tumor necrosis factor (TNF)- $\alpha$  alone for 24 hours a day for 6 days does not result in neuronal death [11]. In contrast, lipopolysaccharide (LPS) or TNF- $\alpha$  stimulation of macrophages induces robust neurotoxicity, which is completely inhibited by the N-methyl D-aspartate (NMDA) receptor antagonist MK-801 [11]. Both the glutaminase inhibitor 6-diazo-5-oxo-L-norleucine (DON), and the gap junction inhibitor carbenoxolone (CBX), also inhibited LPS/TNF- $\alpha$  – induced neurotoxicity by effectively suppressing glutamate production by activated macrophages [11]. Altogether these observations suggest that inflammation alone is not necessarily sufficient for brain injury, but rather it is the combination of inflammatory cytokines/chemokines and excitotoxins (e.g., glutamate) that are most injurious, in other words, immunoexcitotoxicity.

The excitotoxic cascade can be triggered by an excessive release of glutamate from microglia and/or astrocytes, with elevations in nitric oxide (NO), pro-inflammatory prostanooids and generation of a number of reactive oxygen and nitrogen species (ROS/RNS) [6,10,12,13]. NO elevations triggered by activation of inducible NO synthase (iNOS) increase reactions between NO and superoxide with an associated accumulation of very destructive peroxynitrite [13]. Nitrogen and oxygen species also react with membrane lipids resulting in generation of two highly destructive lipid peroxidation products, 4-hydroxynonenal (4-HNE) and acrolein [14]. Peroxynitrite, 4-HNE and acrolein suppress mitochondrial energy production, which dramatically increases the sensitivity to excitotoxicity. Under conditions of reduced energy production, even low levels of glutamate can become excitotoxic [15,16]. It should be noted that glutamate can also activate microglia and enhance cytokine-induced neurodegeneration. Because of this, a self-perpetuating cycle is created in which inflammatory cytokines stimulate the release of glutamate while glutamate, in turn, stimulates the release of inflammatory cytokines. This mutual interaction between inflammatory mediators and the excitatory levels of glutamate further keeps the injured cells locked in a chronic neurodegenerative cycle [17].

### 3. MECHANISTIC BASIS FOR ALUMINUM-INDUCED IMMUNOEXCITOTOXICITY

Studies have shown that microglia and astrocytes are sites of preferential aluminum accumulation and toxic action

[18,19]. Of particular interest is the observation by Suarez-Fernandez *et al.* that chronic aluminum exposure in mixed cultures of astrocytes and neurons results in significant astroglial apoptosis and associated neuronal loss [19]. Given that neurons are dependent on astrocytes for homeostatic control and antioxidant protection and that astrocytes play a critical role in regulating extracellular levels and transport of glutamate, one would not be surprised at this neurotoxic interrelationship. That excitotoxicity secondary to a release of glutamate from the dying astrocytes is the main neurotoxic mechanism is suggested by the observation that neuronal death occurred in cultures containing approximately 10% astrocytes but not in near-pure neuronal cultures containing only 1% astrocytes [19].

Microglia and astrocytes are principal sources of glutamate and immune cytokines in the brain and as such, play a significant role in immunoexcitotoxicity [6,7,10]. Both *in vitro* and *in vivo* studies provide indisputable evidence that aluminum can increase the levels of both pro-inflammatory cytokines and glutamate in the brain [20-25]. Increased expression of these compounds by aluminum in the brain thus likely reflects activation of glial cells.

Interestingly, TNF- $\alpha$ , which is elevated with aluminum exposure, is a key cytokine triggering release of glutamate from microglia, which occurs by up-regulating glutaminase and gap junction hemichannels [22,26]. These observations point to a mechanism by which aluminum can induce immunoexcitotoxicity, a process based on deleterious interactions between inflammatory cytokines (i.e., TNF- $\alpha$ ) and excitotoxins (i.e., glutamate). Indeed, Matyja demonstrated that exposing organotypic cultures of rat hippocampus for 24 hours to a combination of aluminum and glutamate, both in subtoxic concentrations, produced typical excitotoxic lesions, which predominantly consisted of mitochondrial abnormalities [27]. Separately, neither aluminum nor glutamate caused neuronal injury.

### 4. THE ROLE FOR ALUMINUM VACCINE ADJUVANTS IN SEQUENTIAL SYSTEMIC IMMUNE STIMULATION: IMPLICATIONS FOR NEURODEGENERATIVE AND NEURODEVELOPMENTAL DISORDERS

Natural infections are known to activate brain microglia in absence of direct brain infection and both systemic infections and non-infectious systemic immune stimulation have been shown to worsen brain inflammation and neurodegenerative diseases, such as AD and PD [28-30]. Moreover, an early observation linking systemic immune stimulation to changes in behavior and neurological pathology has been called sickness behavior based on the common constellation of neurological symptoms associated with viral illnesses, such as the flu. These can include impaired cognition, poor memory, impaired learning, poor attention, social withdrawal, irritability, reduced food and water intake and depression. Twenty years of research on this phenomenon suggest that it is caused by elevations in pro-inflammatory cytokines and glutamate [31] and is mediated by activated microglia.

Given that the very nature of peripheral immune stimulation can negatively influence brain function, it should not be

considered unreasonable to assume that such outcomes could also occur with administration of aluminum immune adjuvants. Indeed, experimental evidence clearly shows that long-term persistence of aluminum adjuvants in humans results in cognitive dysfunction, affecting visual and verbal memory, as well as executive functions such as attention, working memory and planning [32]. The mode of action of aluminum vaccine adjuvants is still not entirely clear. Recent evidence suggest that the key to developing immunity following vaccination is activation of dendritic cells and that this occurs indirectly *via* immune system stimulation with aluminum-containing adjuvants [33]. Aluminum adjuvants are considered non-TLR type immune adjuvants, acting through local inflammation. It appears that aluminum adjuvants induce chemokines in macrophages, monocytes and granulocytes, which in turn leads to cell recruitment at the injection site and conversion of monocytes into dendritic cells [33]. Moreover, aluminum adjuvants are known to activate some 312 genes, 168 of which play a role in immune activation and inflammation [34]. At least 13 cytokines and chemokines are produced within 4 hours of aluminum adjuvant injection, including interleukin (IL)-1 $\beta$  and IL-5 [35]. Such strong peripheral immune challenges can have an effect on brain immune activation. It is known, for example, that IL-1 $\beta$  is the main stimulus for microglial activation [36].

The mechanism by which systemic activation of brain microglia occurs is critical to understanding the effect of sequential immune stimulation with immune adjuvants, including aluminum. When microglia are first exposed to a disturbance in homeostasis, they may assume a primed state in which their mRNA and membrane receptors are up-regulated, but there is no increased release of cytokines, chemokines, interferons or excitotoxins [37]. Subsequent stimulation will activate these primed microglia with a hyper-responsive reaction, leading to a several fold higher concentration of released pro-inflammatory cytokines, chemokines and the release of three excitotoxins—glutamate, aspartate and quinolinic acid (QUIN) [38].

The greatest aluminum exposure from vaccines occurs during initial vaccinations soon after birth and during early childhood. Should a child follow the recommended vaccine schedule for the United States, they will receive a total of 5 mg of aluminum by 2 years of age from a total of 17 aluminum-adjuvanted pediatric vaccines [2]. Such repetitive and continuous exposure to aluminum from vaccines could induce prolonged activation of microglia and subsequent release of glutamate and pro-inflammatory cytokines. Of special concern is that aluminum in various forms, including adjuvant-aluminum, can accumulate in the brain [39-42].

There is growing evidence that both glutamate and immune cytokines play crucial roles in various aspects of brain development [43,44]. Furthermore, the architectonic development of the nervous system is carefully controlled by a programmed rise and fall of brain glutamate levels and receptor activation during development [43,45]. Perturbations of glutamate and cytokine levels by aluminum early in life could thus be extremely detrimental to normal brain development.

## 5. EVIDENCE FOR ALUMINUM-TRIGGERED IMMUNE AND INFLAMMATORY RESPONSES IN THE CNS

Experimental observations demonstrate that aluminum levels relevant to human exposure from vaccines can directly activate both microglia and astrocytes as well as induce significant inflammatory changes in the brain of rodent species [46,47]. A strong indication that aluminum can activate glial cells in humans comes from an autopsy case of aluminum encephalopathy [48]. In this case a 59-year old woman died following a nine months history of disorientation, memory loss, loss of emotional control, general convulsions and disturbed consciousness. She was a chronic renal patient who took 3 g of hydroxy-aluminum gel per day for 15 years to control her serum phosphorus. X-ray microanalysis demonstrated aluminum deposits in neurons of her cerebral cortex. Notably, neuropathological examination of the brain showed nerve cell atrophy and mild loss with stromal spongiosis, proliferation of astrocytes and microglia in the cerebral cortex, basal ganglia and thalamus. No neurofibrillary tangles (NFT) were seen [48]. The slow evolution of this case suggests a progressive accumulation of aluminum within neurons and glia.

Other aluminum forms such as aluminosilicates have also been reported in diseased brains (i.e., in the cores of senile plaques of AD patients) [49]. Furthermore, Evans *et al.* using purified murine microglia exposed to aluminosilicate particles observed generation of ROS, indicating microglial activation [49].

There is also evidence that systemically administered aluminum from drinking water can specifically activate TNF- $\alpha$  without activating other cytokines. Tsunoda *et al.* exposed male BALB/c mice to aluminum sulfate-containing drinking water ad libitum at concentrations of 0, 5, 25 and 125 ppm aluminum for one month and found significant expression of TNF- $\alpha$  mRNA in the cerebral cortex [22]. Elevations in this cytokine followed a dose-dependent response. The source of the TNF- $\alpha$  was likely from activated microglia. Importantly, these changes occurred at aluminum concentrations commonly encountered in public drinking water. Thus, the study by Tsunoda *et al.* [22] indicates that even very small amounts of aluminum can activate microglia in a pro-inflammatory mode.

Campbell *et al.* observed brain inflammation in animals exposed to aluminum lactate added to their drinking water [25]. The lowest concentration used in their study (0.01nM) was equivalent to that associated with AD and aluminum-containing public drinking water. Unlike the above study, they found elevations in nuclear factor (NF)- $\kappa$ B and IL-1 $\beta$  in the brains of the exposed animals, but no elevation in  $\beta$ -amyloid. Both studies found that systemic exposure to aluminum produced selective inflammation of the brain.

It has also been shown that aluminum in combination with other metals (e.g., copper) additively increases brain inflammation [50]. Interestingly, much like aluminum, other neurotoxic metals commonly found in the environment such as mercury, lead and manganese, have also been shown to activate glial cells, promote both inflammation and oxidative

stress in the brain [51-54]. It is worth emphasizing that all of these metal-induced neurotoxic effects are indicative of excitotoxic processes.

It is of significant concern that low-levels of environmental aluminum are sufficient to induce neurotoxic outcomes [25]. Moreover, experimental evidence shows that aluminum preferentially accumulates in the mitochondria and cell nucleus, which makes this metal very resistant to removal by chelation [55]. Obviously, long-term intracellular persistence of aluminum is likely to exacerbate its toxic effects. The difficulty of removing brain intracellular aluminum will lead to its progressive accumulation over a lifetime, eventually reaching a neurotoxic threshold sufficient to trigger neurodegenerative disease processes [56].

## 6. ALUMINUM ENHANCEMENT OF EXCITOTOXICITY AND IMMUNOEXCITOTOXICITY

Aluminum has been shown to interfere with the action of membrane receptors (i.e., G-protein coupled receptors (GPCRs)), cell signaling pathways, alter DNA integrity and impair mitochondrial function, all of which will have an enhancing effect on both excitotoxicity in general and specifically immunoexcitotoxicity [17].

### 6.1. Aluminum and Energy Metabolism

Aluminum is known to accumulate in the mitochondria and disrupt a number of mitochondrial functions leading to an energy deficit [57, 58], which by itself may not result in sufficient neuronal dysfunction or damage. However, several studies show that reductions in neuronal energy can dramatically amplify excitotoxic injury [59-61]. Impairment of mitochondrial function and energy metabolism by aluminum [57,58,62] would thus be expected to increase the sensitivity of neurons to excitotoxicity, and accelerate and potentiate neuronal damage.

### 6.2. Aluminum and Glutamatergic Neurotransmission

Of particular importance is the effect of aluminum on cell signaling pathways, such as G-proteins, phosphatidylinositol-specific phospholipase C (PI-PLC), protein kinase C and calcium homeostasis [63]. Strunecka *et al.* have shown that aluminum complexed with fluoride can act as a false activator of GPCRs [64]. Further, she proposes that because of the high affinity of fluoride for aluminum, this complex may occur spontaneously in body fluids.

Due to these effects, exposure to aluminofluoride complexes is expected to have profound detrimental consequences on brain functions. This is because metabotropic glutamate receptor (mGluR) signaling, as well as that of many other neurotransmitters, is critically dependent on G-protein receptor systems [65,66]. In general mGluRs enhance excitatory neurotransmission [67] and can therefore worsen excitotoxicity. Under physiological conditions they play an important role in long-term potentiation (LTP) and long-term depression (LTD) and can induce long-lasting changes in neuronal excitability [67]. High levels of mGluR1 expression are seen in the hippocampus [67]. MAPK/ERK and MTOR/p70S6 cell signaling pathways are

particularly important for regulating synaptic plasticity by Group I mGluRs [68].

Group II (mGluR 2/3) and Group III (mGluR 4,6,7,8) are coupled to  $G_{i/o}$  proteins and inhibit adenylyl cyclase, which directly regulates ion channels and other downstream signaling partners *via* liberation of  $G\beta\gamma$  subunits [67]. Group II and III are most often localized pre-synaptically or on pre-terminals of axons and in general inhibit glutamate release [67]. mGluRs are found in virtually every brain region [67], thus adding incredible complexity to glutamate neurotransmission. Both glutamate and mGluRs also play a critical role in brain development [69, 70]. Thus, alterations in the patterns of activation of mGluRs can have a profound effect on eventual brain physiology. By interfering with normal mGluR function, aluminum and especially aluminofluoride complexes, could potentially cause malfunctioning of important brain pathways.

### 6.3. Aluminum and Calcium Homeostasis

Another way in which aluminum may contribute to neuronal injury, particularly associated with AD is by interference with calcium homeostasis, which is known to be perturbed in AD [63,71]. For example, aluminum can delay the closure of voltage-dependent calcium channels and block calmodulin (CaM)-dependent  $Ca^{2+}/Mg^{2+}$ -ATPase, which is responsible for the extrusion of excess intracellular calcium, one of the protective mechanisms against excitotoxicity [72]. El-Rahman exposed male albino rats to aluminum sulfate for 35 days by gavage after which he examined their tissues for aluminum accumulation [24]. Aluminum accumulation as well as aluminum-induced neurotoxic effects were observed in the examined brain sections of the treated animals, and were dose-dependent. Aluminum treated rats also showed a marked increase in brain glutamate levels while their  $\gamma$ -aminobutyric acid (GABA) brain levels were decreased, a condition that maximizes excitotoxic damage. The most significant changes in brain tissue included spongiform changes in neurons, especially within the hippocampus, nuclear deformity, and neurofibrillary degeneration, similar to NFTs in AD [24].

### 6.4. Aluminum and Oxidative Damage

Another link between aluminum toxicity and excitotoxicity is found in the observation by Exley. For example, while aluminum in a non-redox metal, under certain conditions it can act as a pro-oxidant. Moreover, aluminum appears to react with the superoxide radical thus facilitating its destructive potential [73]. Experimental observations also indicate that formation of the superoxide plays a critical step in triggering excitotoxicity-mediated neuronal death through generation of peroxynitrite [10].

Indeed, one of the primary destructive reactions in the excitotoxic cascade is the buildup of peroxynitrite, formed when elevated levels of superoxide react with NO, which is also produced during excitotoxicity [10]. Aluminum could also enhance excitotoxicity by inducing apoptosis of astrocytes, which are thought to be a primary site of aluminum accumulation [19]. Indeed, research evidence shows that, apart from providing a trophic support to neurons, astrocytes also play a crucial role in protecting neurons from excito-

toxic damage, by mediating the clearance of excess glutamate [74]. Aluminum has also been shown to dramatically lower neuronal reduced glutathione levels and astrocytes are the major source of glutathione for neurons [75]. High levels of glutamate also inhibit glutathione production intracellularly by inhibiting the cystine/glutamate antiporter [17]. In addition, aluminum has been shown to impair gap junctional intercellular communication between astrocytes in culture [76].

### 6. 5. Aluminum and Brain Inflammation

Another interesting link to chronic brain inflammation, aluminum and excitotoxicity is the finding that aluminum produces a profound general decrease in nicotine binding involving all brain areas [77]. The nicotinic receptor (alpha7 nicotinic acetylcholine receptor- nAChR) plays an important role in dampening brain inflammation and interference with cholinergic neurotransmission can increase brain inflammation [78]. This may be a major mechanism linking aluminum accumulations in the AD brain and chronic inflammation that goes beyond the neurotransmitter functions of acetylcholine.

Taken together, all of the above experimental observations indicate that by promoting oxidative damage and inflammation in the CNS, exacerbating excitotoxic damage by (i) increasing the levels of excitotoxic mediators and (ii) impeding their clearance, aluminum can both trigger and promote neuronal injury. Aluminum-induced microglial/astrocyte-mediated immunoexcitotoxicity combined with its direct neurotoxic effects, makes this element a strong candidate for at least enhancing neurodegeneration associated with such disorders as AD, PD, ALS, HD, MS, viral encephalopathies and CTE. Notably, all of these diseases have been previously linked to over-active glia [3,5-7,17,79].

### 7. ABSORPTION AND DISTRIBUTION OF BIO-AVAILABLE ALUMINUM

When assessing aluminum's potential toxicity, one must also consider absorption and distribution. While absorption from foods is considered to be quite small, under certain conditions this can be increased substantially. For example, organic acids and some amino acids increase aluminum absorption significantly. Increasing absorption is as follows: aluminum citrate > aluminum tartrate > aluminum gluconate > aluminum lactate > aluminum glutamate > AlCl<sub>3</sub>, AlSO<sub>4</sub>, aluminum nitrate [80].

Two forms of aluminum are of special concern: aluminum-L-glutamate and nanoscaled aluminum, both of which have high absorption from the gut and passage into the brain, as well as higher toxicity profiles than aluminum alone. Adding to this concern is the fact that glutamate, both as a food additive and naturally occurring in foods, is common in the Western diet. Deloncle *et al.* injected Al-L-glutamate subcutaneously and i.v. for 5 weeks and demonstrated a significant increase in aluminum content in several areas of the animals' brain, including the hippocampus, occipito-parietal cortex, cerebellum and striatum [81]. It is of considerable interest that 50% of the animals given subcutaneous injections of aluminum glutamate developed neurological disturbances such as trembling, equilibrium disturbances and convulsions,

leading to death. This is suggestive of an excitotoxic effect. Supporting this conclusion, Deloncle *et al.* found significant elevations in glutamate in the occipito-parietal cortex of Al-L-glutamate-treated animals. These observations suggest that the Al-L-glutamate complex is capable of crossing the BBB.

Nanoscaled aluminum has higher absorption rates than naturally found aluminum. Li *et al.* compared nanoscaled aluminum oxide with non-nanoscaled aluminum oxide in terms of their ability to activate microglia and astrocytes and found that nanoscaled aluminum oxide produced significantly greater activation of glial cells than did non-nanoscaled aluminum oxide [47]. Of note, nanoparticulate aluminum compounds are being used in a growing number of products as well as vaccines [82].

It is important to know what proportion of absorbed aluminum reaches the brain in comparison to other organs. To answer this question, Flarend *et al.* [83] injected New Zealand White rabbits intramuscularly with <sup>26</sup>Al radiolabeled aluminum hydroxide and aluminum phosphate and traced aluminum distribution at 28 days post-injection in blood and urine samples as well as tissues, by utilizing accelerator mass spectrometry. The Al isotope appeared in the first blood sample at one hour for both adjuvants, but levels were 3x higher for the aluminum phosphate than the aluminum hydroxide. Tissue distribution profiles were the same for both compounds (kidney>spleen>liver>heart>lymph nodes>brain). Even though brain levels were low following a single injection, children are receiving multiple injections repeatedly during early life in addition to a lifetime of aluminum exposure from other environmental sources. Furthermore, substantial evidence shows that bio-available aluminum tends to accumulate in the brain over a lifetime [56,63].

### 8. CONCLUSION

The purpose of this review was to describe two mechanism linking aluminum to neurodegenerative processes and neurological dysfunction—systemic activation of CNS microglia and immunoexcitotoxicity. The first of these mechanisms, referred to as sickness behavior, has been extensively studied and provides both, a direct and indirect link between systemic immune activation, activation of CNS microglia and abnormal neurological symptoms. In an analogous fashion, immune stimulation by aluminum adjuvants has also been shown to result in adverse neurological outcomes *via* activation of microglia, which are the brain's resident immune cells. Once activated, microglia become the main source of both pro-inflammatory immune cytokines and excitotoxins such as glutamate. It is the interaction of cytokines and glutamate receptors that leads to immunoexcitotoxicity. Other than activating glial cells, aluminum also directly impairs a number of energy related enzymes, promotes brain inflammation, oxidative damage, reduces the levels of brain antioxidants (i.e., glutathione) and disturbs calcium homeostasis. All of these effects will amplify immunoexcitotoxic damage. In the immature and developing brain, immunoexcitotoxicity might lead to a number of neurodevelopmental conditions, such as autism spectrum disorders and seizures. In the mature, and especially the aging brain, these mechanisms can lead to progressive neurodegeneration, as seen with AD, PD and ALS.

There is suggestive evidence that microglia can become stuck in a neurodestructive mode over long periods of time [5,17,79,84,85]. It may be that rather than chronic activation, it is the repeated stimulation of primed microglia that leads to chronic, progressive neurodegeneration. The presence of aluminum deposits within the neurons and glial cells could act as a continuous stimulus for immunoexcitotoxicity. There is now sufficient evidence from a great number of studies to call for a re-evaluation of the use of aluminum additives for human consumption or as immune adjuvants.

## 9. ABBREVIATIONS

AD	=	Alzheimer's disease
ALS	=	Amyotrophic lateral sclerosis
CaM	=	Calmodulin
CNS	=	Central nervous system
GABA	=	$\gamma$ -Aminobutyric acid
GPCR	=	G-protein coupled receptor
4-HNE	=	4-hydroxynonenal
IL	=	Interleukin
iNOS	=	Inducible nitric oxide synthase
LPS	=	Lipopolysaccharide
LTD	=	Long-term depression
LTP	=	Long-term potentiation
mGluR	=	Metabotropic glutamate receptor
MHC	=	Major histocompatibility complex class
MS	=	Multiple sclerosis
NADPH	=	Nicotinamide adenine dinucleotide phosphate
NF- $\kappa$ B	=	Nuclear factor kappa B
NFT	=	Neurofibrillary tangles
NMDA	=	N-methyl D-aspartate
NO	=	Nitric oxide
QUIN	=	Quinolinic acid
PD	=	Parkinson's disease
PI-PLC	=	Phosphatidylinositol-specific phospholipase
PRR	=	Pattern recognition receptors
RNS	=	Reactive nitrogen species
ROS	=	Reactive oxygen species
TLR	=	Toll-like receptor

## CONFLICT OF INTEREST

No conflict of interest is declared.

## REFERENCES

- [1] Exley, C.; House, E. Aluminium in the human brain. *Monatsh. Chem.*, **2011**, *142*, 357-363.
- [2] Tomljenovic, L.; Shaw, C.A. Aluminum vaccine adjuvants: are they safe? *Curr. Med. Chem.*, **2011**, *18*, 2630-2637.
- [3] Blaylock, R.L. A possible central mechanism in Autism Spectrum Disorders, Part 2: Immunoexcitotoxicity. *Alter. Ther. Health. Med.*, **2009**, *15*, 60-67.
- [4] Lawson, L.J.; Perry, V.H.; Dri, P.; Gordon, S. Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. *J. Neurosci.*, **1990**, *39*, 151-170.
- [5] Perry, V.H.; Nicoll, J.A.; Holmes, C. Microglia in neurodegenerative disease. *Nat. Rev. Neurol.*, **2010**, *6*, 193-201.
- [6] Block, M.L.; Zecca, L.; Hong, J.S. Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. *Nat Rev Neurosci.*, **2007**, *8*, 57-69.
- [7] Harry, G.J.; Kraft, A.D. Neuroinflammation and microglia: considerations and approaches for neurotoxicity assessment. *Expert. Opin. Drug. Metab. Toxicol.*, **2008**, *4*, (10), 1265-1277.
- [8] Kreutzberg, G.W. Microglia: a sensor for pathological events in the CNS. *Trends Neurosci.*, **1996**, *19*, (8), 312-31.
- [9] van Noort, J.M.; Bsibsi, M. Toll-like receptors in the CNS: implications for neurodegeneration and repair. *Prog. Brain. Res.*, **2009**, *175*, 139-148.
- [10] Mander, P.; Brown, G.G.; Activation of microglial NADPH oxidase is synergistic with glial iNOS expression in inducing neuronal death: a dual-key mechanism of inflammatory neurodegeneration. *J. Neuroinflammation*, **2005**, *2*, doi: 10.1186/1742-2094-2-20
- [11] Yawata, I.; Takeuchi, H.; Doi, Y.; Liang, J.; Mizuno, T.; Suzumura, A. Macrophage-induced neurotoxicity is mediated by glutamate and attenuated by glutaminase inhibitors and gap junction inhibitors. *Life Sci.*, **2008**, *82*, (21-22): 1111-1116.
- [12] Brown, G.C.; Neher, J.J. Inflammatory neurodegeneration and mechanisms of microglial killing of neurons. *Mol. Neurobiol.*, **2010**, *41*, (2-3), 242-247.
- [13] Poderoso, J.J. The formation of peroxynitrite in the applied physiology of mitochondrial nitric oxide. *Arch. Biochem. Biophys.*, **2009**, *484*, (2), 214-220.
- [14] Nam, D.T.; Arseneault, M.; Murthy, V.; Ramassamy, C. Potential role of acrolein in neurodegeneration and in Alzheimer's disease. *Curr. Mol. Pharmacol.*, **2010**, *3*, (2), 66-78.
- [15] Izumi, Y.; Zorumski, C.F. Neuroprotective effects of pyruvate following NMDA-mediated excitotoxic insults in hippocampal slices. *Neurosci. Lett.*, **2010**, *478*, (3), 131-135.
- [16] Beal, M.F. Does impairment of energy metabolism result in excitotoxic neuronal death in neurodegenerative illnesses? *Ann. Neurol.*, **1992**, *31*(2), 119-130.
- [17] Blaylock, R.L.; Maroon, J. Immunoexcitotoxicity as a central mechanism in chronic traumatic encephalopathy—a unifying hypothesis. *Surg. Neurol. Int.* **2011**, *2*, 107, doi: 10.4103/2152\_7806.83391.
- [18] Platt, B.; Fiddler, G.; Riedel, G.; Henderson, Z. Aluminium toxicity in the rat brain: histochemical and immunocytochemical evidence. *Brain. Res. Bull.*, **2001**, *55*, (2), 257-267.
- [19] Suarez-Fernandez, M.B.; Soldado, A.B.; Sanz-Medel, A.; Vega, J.A.; Novelli, A.; Fernandez-Sanchez, M.T. Aluminium-induced degeneration of astrocytes occurs *via* apoptosis and results in neuronal death. *Brain. Res.*, **1999**, *835*, (2), 125-136.
- [20] Johnson, V.J.; Sharma, R.P. Aluminium disrupts the pro-inflammatory cytokine/neurotrophin balance in primary brain rotation-mediated aggregate cultures: possible role in neurodegeneration. *Neurotoxicology*, **2003**, *24*, (2), 261-268.
- [21] Nayak, P.; Chatterjee, A.K.; Effects of aluminum exposure on brain glutamate and GABA systems: an experimental study in rats. *Food. Chem. Toxicol.*, **2001**, *39*, (12), 1285-1289.
- [22] Tsunoda, M.; Sharma, R.P. Modulation of tumor necrosis factor alpha expression in mouse brain after exposure to aluminum in drinking water. *Arch. Toxicol.*, **1999**, *73*, (8-9), 419-426.
- [23] El-Rahman, S.S. Neuropathology of aluminum toxicity in rats (glutamate and GABA impairment). *Pharmacol. Res.*, **2003**, *47*, (3), 189-194.
- [24] Bondy, S.C. The neurotoxicity of environmental aluminum is still an issue. *Neurotoxicology*, **2010**, *31*, (5), 575-581.
- [25] Campbell, A.; Becaria, A.; Lahiri, D.K.; Sharman, K.; Bondy, S.C. Chronic exposure to aluminum in drinking water increases inflammatory parameters selectively in the brain. *J. Neurosci. Res.*, **2004**, *75*, (4), 565-572.
- [26] Takeuchi, H.; Jin, S.; Wang, J.; Zhang, G.; Kawanokuchi, J.; Kuno, R.; Sonobe, Y.; Mizuno, T.; Suzumura, A. Tumor necrosis factor-

- alpha induces neurotoxicity via glutamate release from hemichannels of activated microglia in an autocrine manner. *J. Biol. Chem.*, **2006**, *281*, (30), 21362-21368.
- [27] Matyja, E. Aluminum enhances glutamate-mediated neurotoxicity in organotypic cultures of rat hippocampus. *Folia Neuropathol.*, **2000**, *38*, (2), 47-53.
- [28] Perry, V.H. The influence of systemic inflammation on inflammation in the brain: Implications from chronic neurodegenerative diseases. *Brain. Behav. Immun.* **2004**, *18*, (5), 407-413.
- [29] Cunningham, C.; Campion, S.; Lunnon, K.; Murray, C.L.; Woods, J.F.; Deacon, R.M.; Rawlins, J.N.; Perry, V.H. Systemic inflammation induces acute behavioral and cognitive changes and accelerates neurodegenerative disease. *Biol. Psychiatry*, **2009**, *65*, (4), 304-312.
- [30] Cunningham, C.; Campion, S.; Teeling, J.; Felton, L.; Perry, V.H.; The sickness behavior and CNS inflammatory mediator profile induced by systemic challenge of mice with synthetic double-stranded RNA (Poly I:C). *Brain. Behav. Immun.*, **2007**, *21*, (4), 490-502.
- [31] Dantzer, R.; Kelley, K.W. Twenty years of research on cytokine-induced sickness behavior. *Brain. Behav. Immun.*, **2007**, *21*, (2), 153-160.
- [32] Couette, M.; Boisse, M.F.; Maison, P.; Brugieres, P.; Cesaro, P.; Chevalier, X.; Gherardi, R.K.; Bachoud-Levi, A.C.; Authier, F.J. Long-term persistence of vaccine-derived aluminum hydroxide is associated with chronic cognitive dysfunction. *J. Inorg. Biochem.*, **2009**, *103*, (11), 1571-1578.
- [33] Exley, C.; Siesjo, P.; Eriksson, H. The immunobiology of aluminium adjuvants: how do they really work? *Trends. Immunol.*, **2010**, *31*, (3), 103-109.
- [34] Mosca, F.; Tritto, E.; Muzzi, A.; Monaci, E.; Bagnoli, F.; Iavarone, C.; O'Hagan, D.; Rappuoli, R.; De Gregorio, E. Molecular and cellular signatures of human vaccine adjuvants. *Proc. Natl. Acad. Sci. U.S.A.*, **2008**, *105*, (30), 10501-10506.
- [35] McKee, A.S.; Munks, M.W.; MacLeod, M.K.; Fleenor, C.J.; Van Rooijen, N.; Kappler, J.W.; Marrack, P. Alum induces innate immune responses through macrophage and mast cell sensors, but these sensors are not required for alum to act as an adjuvant for specific immunity. *J. Immunol.*, **2009**, *183*, (7), 4403-4414.
- [36] Rothwell, N.; Allan, S.; Toulmond, S. The role of interleukin 1 in acute neurodegeneration and stroke: pathophysiological and therapeutic implications. *J. Clin. Invest.*, **1997**, *100*, (11), 2648-2652.
- [37] Town, T.; Nikolic, V.; Tan, J. The microglial "activation" continuum: from innate to adaptive responses. *J. Neuroinflammation*, **2005**, *2*, 24.
- [38] Takeuchi, H. Neurotoxicity by microglia: mechanisms and potential therapeutic strategy. *Clin. Exp. Neuroimmunol.*, **2010**, *1*, 12-21.
- [39] Perl, D.P.; Moalem, S. Aluminum and Alzheimer's disease, a personal perspective after 25 years. *J. Alzheimers. Dis.*, **2006**, *9*, (3 Suppl), 291-300.
- [40] Walton, J.R. Aluminum in hippocampal neurons from humans with Alzheimer's disease. *Neurotoxicology*, **2006**, *27*, (3), 385-394.
- [41] Walton, J.R. An aluminum-based rat model for Alzheimer's disease exhibits oxidative damage, inhibition of PP2A activity, hyperphosphorylated tau, and granulovacuolar degeneration. *J. Inorg. Biochem.*, **2007**, *101*, (9), 1275-1284.
- [42] Redhead, K.; Quinlan, G.J.; Das, R.G.; Gutteridge, J.M. Aluminium-adjuvanted vaccines transiently increase aluminium levels in murine brain tissue. *Pharmacol. Toxicol.*, **1992**, *70*, (4), 278-280.
- [43] Matsugami, T.R.; Tanemura, K.; Mieda, M.; Nakatomi, R.; Yamada, K.; Kondo, T.; Ogawa, M.; Obata, K.; Watanabe, M.; Hashikawa, T.; Tanaka, K. From the Cover: Indispensability of the glutamate transporters GLAST and GLT1 to brain development. *Proc. Natl. Acad. Sci. U.S.A.*, **2006**, *103*, (32), 12161-12166.
- [44] Garay, P.A.; McAllister, A.K. Novel roles for immune molecules in neural development: implications for neurodevelopmental disorders. *Front. Synaptic. Neurosci.*, **2010**, *2*, 136.
- [45] Komuro, H.; Rakic, P. Modulation of neuronal migration by NMDA receptors. *Science*, **1993**, *260*, (5104), 95-97.
- [46] Shaw, C.A.; Petrik, M.S. Aluminum hydroxide injections lead to motor deficits and motor neuron degeneration. *J. Inorg. Biochem.*, **2009**, *103*, (11), 1555-62.
- [47] Li, X.; Zheng, H.; Zhang, Z.; Li, M.; Huang, Z.; Schluesener, H.J.; Li, Y.; Xu, S. Glia activation induced by peripheral administration of aluminum oxide nanoparticles in rat brains. *Nanomed.*, **2009**, *5*, (4), 473-479.
- [48] Shirabe, T.; Irie, K.; Uchida, M. Autopsy case of aluminum encephalopathy. *Neuropathology*, **2002**, *22*, (3), 206-210.
- [49] Evans, P.H.; Yano, E.; Klinowski, J.; Peterhans, E. Oxidative damage in Alzheimer's dementia, and the potential etiopathogenic role of aluminosilicates, microglia and micronutrient interactions. *EXS.*, **1992**, *62*, 178-189.
- [50] Becaria, A.; Lahiri, D.K.; Bondy, S.C.; Chen, D.; Hamadeh, A.; Li, H.; Taylor, R.; Campbell, A. Aluminum and copper in drinking water enhance inflammatory or oxidative events specifically in the brain. *J. Neuroimmunol.*, **2006**, *176*, (1-2): 16-23.
- [51] Yin, Z.; Milatovic, D.; Aschner, J.L.; Syversen, T.; Rocha, J.B.; Souza, D.O.; Sidoryk, M.; Albrecht, J.; Aschner, M. Methylmercury induces oxidative injury, alterations in permeability and glutamine transport in cultured astrocytes. *Brain. Res.*, **2007**, *1131*, (1), 1-10.
- [52] Charleston, J.S.; Body, R.L.; Bolender, R.P.; Mottet, N.K.; Vahter, M.E.; Burbacher, T.M. Changes in the number of astrocytes and microglia in the thalamus of the monkey *Macaca fascicularis* following long-term subclinical methylmercury exposure. *Neurotoxicology*, **1996**, *17*, (1), 127-138.
- [53] Monnet-Tschudi, F.; Zurich, M.G.; Boschat, C.; Corbaz, A.; Honegger, P. Involvement of environmental mercury and lead in the etiology of neurodegenerative diseases. *Rev. Environ. Health*, **2006**, *21*, (2), 105-117.
- [54] Burton, N.C.; Guilarte, T.R. Manganese neurotoxicity: lessons learned from longitudinal studies in nonhuman primates. *Environ. Health. Perspect.*, **2009**, *117*, (3), 325-332.
- [55] Kruck, T.P.; Cui, J.G.; Percy, M.E.; Lukiw, W.J. Molecular shuttle chelation: the use of ascorbate, desferrioxamine and Feralex-G in combination to remove nuclear bound aluminum. *Cell. Mol. Neurobiol.*, **2004**, *24*, (3), 443-459.
- [56] Walton, J.R.; Wang, M.X. APP expression, distribution and accumulation are altered by aluminum in a rodent model for Alzheimer's disease. *J. Inorg. Biochem.*, **2009**, *103*, (11), 1548-1554.
- [57] Mohan, N.; Alleyne, T.; Adogwa, A. The effects of ingested aluminum on brain cytochrome oxidase activity. *West. Indian Med. J.*, **2009**, *58*, (5), 422-427.
- [58] Kumar, V.; Bal, A.; Gill, K.D. Impairment of mitochondrial energy metabolism in different regions of rat brain following chronic exposure to aluminium. *Brain. Res.*, **2008**, *1232*, 94-103.
- [59] Ludolph, A.C.; Riepe, M.; Ullrich, K. Excitotoxicity, energy metabolism and neurodegeneration. *J. Inherit. Metab. Dis.*, **1993**, *16*, (4), 716-723.
- [60] Storey, E.; Hyman, B.T.; Jenkins, B.; Brouillet, E.; Miller, J.M.; Rosen, B.R.; Beal, M.F. 1-Methyl-4-phenylpyridinium produces excitotoxic lesions in rat striatum as a result of impairment of oxidative metabolism. *J. Neurochem.*, **1992**, *58*, (5), 1975-1978.
- [61] Greenamyre, J.T. Neuronal bioenergetic defects, excitotoxicity and Alzheimer's disease: "use it and lose it". *Neurobiol. Aging.*, **1991**, *12*, (4), 334-336; discussion 352-335.
- [62] Silva, V.S.; Goncalves, P.P. The inhibitory effect of aluminum on the (Na<sup>+</sup>/K<sup>+</sup>) ATPase activity of rat brain cortex synaptosomes. *J. Inorg. Biochem.*, **2003**, *97*, (1), 143-150.
- [63] Tomljenovic, L. Aluminum and Alzheimer's disease: after a century of controversy, is there a plausible link? *J. Alzheimers Dis.*, **2011**, *23*, (4), 567-598.
- [64] Strunecka, A.; Strunecky, O.; Patocka, J. Fluoride plus aluminum: useful tools in laboratory investigations, but messengers of false information. *Physiol. Res.*, **2002**, *51*, (6), 557-564.
- [65] Morris, A.J.; Malbon, C.C. Physiological regulation of G protein-linked signaling. *Physiol. Rev.*, **1999**, *79*, (4), 1373-1430.
- [66] Odagaki, Y.; Nishi, N.; Koyama, T. Functional coupling between metabotropic glutamate receptors and G proteins in rat brain membranes. *Eur. J. Pharmacol.*, **1996**, *300*, (1-2), 151-154.
- [67] Cosgrove KE, Galvan EJ, Barrionuevo G, Meriney SD. mGluRs modulate strength and timing of excitatory transmission in hippocampal area CA3. *Mol Neurobiol* 2011; *44*(1): 93-101.
- [68] Page G, Khidir FA, Pain S, Barrier L, Fauconneau B, Guillard O, Piriou A, Hugon J. Group 1 metabotropic glutamate receptors activate the p70S6 kinase via both mammalian target of rapamycin (mTOR) and extracellular signal-regulated kinase (ERK1/2) signaling pathways in rat striatal and hippocampal synaptoneuroosomes. *Neurochem Int* 2006; *49*(4): 413-421.

- [69] Griesmaier, E.; Keller, M. Glutamate receptors - Prenatal insults, long-term consequences. *Pharmacol. Biochem. Behav.*, **2011**, doi: 10.1016/j.pbb.2011.04.011
- [70] Schlett, K. Glutamate as a modulator of embryonic and adult neurogenesis. *Curr. Top. Med. Chem.* **2006**, *6*, (10), 949-960.
- [71] LaFerla, F.M. Calcium dyshomeostasis and intracellular signalling in Alzheimer's disease. *Nat. Rev. Neurosci.*, **2002**, *3*, (11), 862-872.
- [72] Exley, C. A molecular mechanism of aluminium-induced Alzheimer's disease? *J Inorg Biochem.* **1999**, *76*, (2), 133-140.
- [73] Exley, C. The pro-oxidant activity of aluminum. *Free Rad. Biol. Med.*, **2004**, *36*, (3), 380-387.
- [74] Aremu, D.A.; Meshitsuka, S. Some aspects of astroglial functions and aluminum implications for neurodegeneration. *Brain Res Rev.* **2006**, *52*, (1), 193-200.
- [75] Murakami, K.; Yoshino, M. Aluminum decreases the glutathione regeneration by the inhibition of NADP-isocitrate dehydrogenase in mitochondria. *J. Cell. Biochem.*, **2004**, *93*, (6), 1267-1271.
- [76] Theiss, C.; Meller, K. Aluminum impairs gap junctional intercellular communication between astroglial cells *in vitro*. *Cell. Tissue Res.*, **2002**, *310*, (2), 143-154.
- [77] Gulya, K.; Rakonczay, Z.; Kasa, P. Cholinotoxic effects of aluminum in rat brain. *J. Neurochem.*, **1990**, *54*, (3), 1020-1026.
- [78] Tyagi, E.; Agrawal, R.; Nath, C.; Shukla, R. Inhibitory role of cholinergic system mediated *via* alpha7 nicotinic acetylcholine receptor in LPS-induced neuro-inflammation. *Innate. Immun.*, **2009**, *16*, (1), 3-13.
- [79] Block, M.L.; Hong, J.S. Chronic microglial activation and progressive dopaminergic neurotoxicity. *Biochem. Soc. Trans.*, **2007**, *35*, (Pt 5), 1127-1132.
- [80] Cunat, L.; Lanhers, M.C.; Joyeux, M.; Burnel, D. Bioavailability and intestinal absorption of aluminum in rats: effects of aluminum compounds and some dietary constituents. *Biol. Trace. Elem. Res.*, **2000**, *76*, (1), 31-55.
- [81] Deloncle, R.; Guillard, O.; Huguet, F.; Clanet, F. Modification of the blood-brain barrier through chronic intoxication by aluminum glutamate. Possible role in the etiology of Alzheimer's disease. *Biol. Trace. Elem. Res.*, **1995**, *47*, (1-3), 227-233.
- [82] Frey, A.; Neutra, M.R.; Robey, F.A. Peptomer aluminum oxide nanoparticle conjugates as systemic and mucosal vaccine candidates: synthesis and characterization of a conjugate derived from the C4 domain of HIV-1MN gp120. *Bioconjug. Chem.*, **1997**, *8*, (3), 424-433.
- [83] Flarend, R.E.; Hem, S.L.; White, J.L.; Elmore, D.; Suckow, M.A.; Rudy, A.C.; Dandashli, E.A. *In vivo* absorption of aluminium-containing vaccine adjuvants using 26Al. *Vaccine*, **1997**, *15*, (12-13), 1314-1318.
- [84] Pardo, C.A.; Vargas, D.L.; Zimmerman, A.W. Immunity, neuroglia and neuroinflammation in autism. *Int. Rev. Psychiatry*, **2005**, *17*, (6), 485-495.
- [85] Yasuda, Y.; Shinagawa, R.; Yamada, M.; Mori, T.; Tateishi, N.; Fujita, S. Long-lasting reactive changes observed in microglia in the striatal and substantia nigral of mice after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Brain. Res.*, **2007**, *1138*, 196-202.