Molecular and Genetic Medicine

Beecham and Seneff, J Mol Genet Med 2015, 9:4
http://dx.doi.org/10.4172/1747-0862.1000187

Review Article Open Access

The Possible Link between Autism and Glyphosate Acting as Glycine Mimetic - A Review of Evidence from the Literature with Analysis

Beecham JE and Stephanie Seneff *

Computer Science and Artificial Intelligence Laboratory, Massachusetts Institute of Technology, Cambridge MA 02139, USA

***Corresponding author:** Stephanie Seneff, Computer Science and Artificial Intelligence Laboratory, Massachusetts Institute of Technology, Cambridge MA 02139, USA, E-mail: seneff@csail.mit.edu

Received date: August 26, 2015, **Accepted date:** October 06, 2015, **Published date:** October 13, 2015

Abstract

The causes of autism spectrum disorder (ASD) are not well understood. Only a minority of cases are explainable by specific abnormalities in DNA sequence, whereas the majority are widely assumed to be linked to epigenetic effects, and/or likely impacted by environmental factors. Here, we postulate autism causation via environmental and/or dietary sourced toxin acting intermittently in utero on human fetuses to disrupt neurodevelopment in a non-dose dependent manner. Our theory is informed by a mini-review and correlation of selected studies from the research literature related to autism, including radiologic, anatomic, metabolic, neurodevelopmental, pharmacologic and MRI studies. In reviewing and analyzing evidence, we focus on data supporting interaction of the theorized harmful glycine mimetic at one or more of the following calcium inflow regulatory factors for neurons: the N-methyl D-aspartate (NMDA) receptor, the glycine receptor (GlyR) and/or the glycine transporter protein 1 (GlyT1).

We postulate this harmful glycine mimetic to act by exerting a direct molecular disruption to calcium regulatory factors for neurons. This disruption appears to occur in a time sensitive, rather than a strictly dose-dependent manner, leading to haphazard disorganizations of the normally carefully choreographed steps of early neuronal migration. Within this analysis, we find support for the contention that a strong candidate for the putative harmful glycine mimetic is glyphosate, the active ingredient in the pervasive herbicide Roundup®. In addition to glyphosate's molecular similarity to glycine, glyphosate is known to have a propensity to avidly bind minerals such as manganese and magnesium, which minerals are implicated in the normal functioning of several neuronal calcium inflow regulatory factors. Our theory highlights areas deserving of further study.

Introduction

Since 1980, the number of children known to have autism spectrum disorder (ASD) has increased dramatically. However this increase is thought to be at least partly due to changes in diagnostic practices [1]. The risk of autism is known to be associated with advanced paternal age and with diabetes in the mother during pregnancy [2]. There is a well-known male preponderance in ASD cases [1]. Although autism is believed, by many experts, to be caused by inherited factors, no single specific DNA sequence alteration has been found to explain more than a minority of the cases.

A recent report by Yuen et al. [3] has raised questions about the subject of heritable gene defects in regard to autism causation. Because autism often runs in families, experts had assumed that siblings with the disorder were inheriting the same autism-predisposing genes from their parents. It now appears this may not be so. These researchers sequenced, from 85 families each having two children affected by autism, 340 whole genomes, including from 170 individuals with ASD. They found that the majority of autism sibling pairs (69 percent) had little to no overlap in abnormal genes. Sibling pairs shared the same autism-associated gene changes less than one third of the time (31 percent). The majority of autism-affected sibling pairs (69 percent) had little to no overlap in the gene variations thought to contribute to autism.

This raises the question whether children are developing autism from an environmental or dietary toxin for which the DNA alterations signal presence of the toxin, but wherein the genetic changes are not conferring all the autism-causing action. The possibility exists that the putative toxin is also, or perhaps principally, causing damage by interacting directly within the neurodevelopmental molecular structures and pathways of the developing brain. A candidate molecule, as will be discussed in more detail later, is glyphosate. It is noteworthy that, in a study by Koller et al. [4], human buccal epithelial cells from cell line TR146 were exposed to glyphosate alone in one test, and separately in another test using only the glyphosate-containing formulation known as Roundup®. Each test was at concentrations representing a 450-fold dilution of concentrations used in spraying crops in agriculture. Separately, in each of the two test exposures, DNA damage was found to occur in the exposed epithelial cells.

Experts, when ascribing causation in autism, often invoke epigenetic changes, citing unspecified reactions, or perhaps environmental influences [5]. Such epigenetic changes, while not modifying the DNA sequence code, are nevertheless thought to be heritable and causative, affecting early/fetal neurodevelopment [6]. Environmental causes thus have not been ruled out as causative or contributory in autism [7], and exposure to air pollution, especially particulates and heavy metals, are acknowledged to perhaps increase the risk of autism [8]. Furthermore, some cases of autism have been

strongly associated with agents that cause birth defects [9], and these teratogens are widely believed to have their greatest effect within the first 8 weeks from conception.

Rzhetsky et al. [10] reported in 2014 on the results of their analysis of geographic spatial incidence patterns of ASD and Intellectual Disability (ID) as reflected in insurance claims datasets representing nearly 1/3 of the entire US population. They used "the rate of congenital malformations of the reproductive system as a surrogate for environmental exposure of parents to unmeasured developmental risk factors, including toxins" because "70 to 80% of male congenital malformations of the reproductive system have no clear genetic causes. Instead, they appear to be driven by specific environmental insults…" They found that, "Adjusted for gender, ethnic, socioeconomic, and geopolitical factors, the ASD incidence rates were strongly linked to population-normalized rates of congenital malformations of the reproductive system in males (an increase in ASD incidence by 283% for every percent increase in incidence of malformations, 95% CI: [91%, 576%], p<6×10-5)". Their conclusions state that, in attempting to ascribe causation in cases of autism, in addition to evaluating possible chromosomal DNA sequence alterations, "detailed documentation of environmental factors should be recorded and used… and failure to do so risks omitting important information."

To clarify theories of causation in autism, a review of selected studies was conducted and correlated, as described below.

Selected Magnetic Resonance Imaging (MRI) Studies Related to Autism

Haari et al. [11] reviewed structural MRI scans from 539 high functioning adults with autism and compared the images to structural MRI scans from 573 controls. These researchers concluded there was no evidence within their MRI images for significant between-group differences in any measures of gross anatomy or in specific brain regions, including several which had previously been implicated in anatomic studies of autism spectrum disorder (ASD), including the amygdala, hippocampus, most segments of the corpus callosum and the cerebellum.

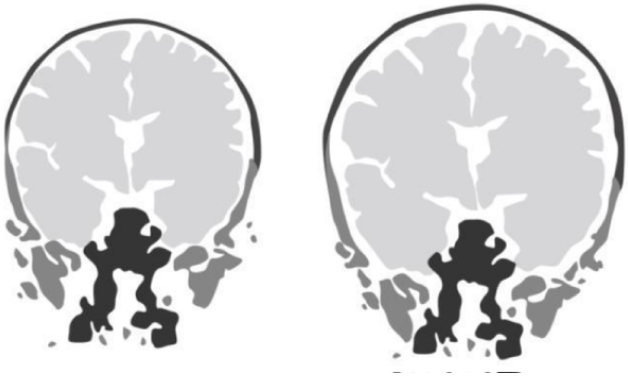

Figure 1: Modified from Shen [12]: Illustration of MRI pattern from child (left) not destined to develop autism compared to same age child (right) destined to develop autism. Note increased brain size and CSF accumulation on the right side image.

By contrast, an MRI study by Shen et al. [12] reported it was possible to fairly reliably separate the MRI brain images of children who will go on to develop autism from controls (Figure 1). Fifty-five infants (33 'high-risk' infants having an older sibling with autism spectrum disorder and 22 'low-risk' infants having no relatives with autism spectrum disorder) were imaged at 6-9 months; 43 of these (27 high-risk and 16 low-risk) were imaged at 12-15 months; and 42 (26 high-risk and 16 low-risk) were imaged again at 18-24 months. The children were followed to determine which developed autism. The presence in the scans of increased brain volume and increased cerebrospinal fluid (CSF) accumulation was reported as predictive of subsequent development of autism, according to the researchers.

Glial Cells in Autism

Background: The central nervous system is comprised of neurons and three basic types of glial cells: microglia, astrocytes, and oligodendrocytes. One primary function of glial cells is to support, protect, and nurture the neurons, a process that is essential for neural plasticity and stability. Microglia migrate to the central nervous system (CNS) during prenatal development and are the resident immune cells in both the brain and spinal cord. Microglia maintain vigilance for any type of toxic challenge to the CNS, including injury, infection, and ischemia. They respond with activation, into a pro-inflammatory state for example, in response to neuronal cell injury or death.

MRI study of pattern of glial cell activation in autism

Suzuki et al. [13] studied microglial activation using MRI images in a case-control study design with positron emission tomography and a radiotracer for microglia; [11C](R)-(1-[2-chrorophynyl]-N-methyl-N-[1-methylpropyl]-3 isoquinoline carboxamide) ([11C](R)-PK11195) (Figure 2). They recruited study participants from the area of Hamamatsu, Japan. Their study population consisted of Japanese citizens, twenty men with ASD (age range, 18-31 years; mean [SD] IQ, 95.9 [16.7]) and 20 age- and IQ-matched healthy men as controls.

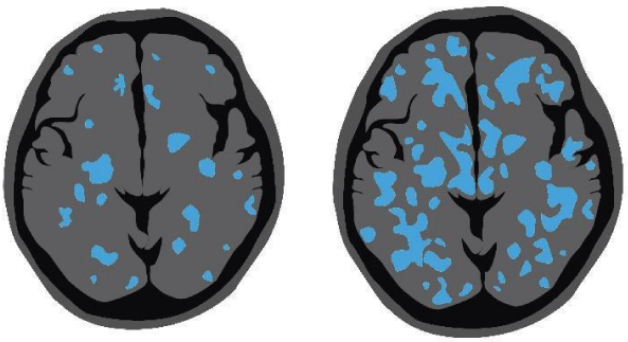

Figure 2: Modified from Suzuki [13]: Illustration of MRI image pattern of control brain (left), and autism brain (right); both have same pattern of activated microglia, but degree varies, increased in autism brain.

Diagnosis of autism spectrum disorder (ASD) was made in accordance with the Autism Diagnostic Observation Schedule and the Autism Diagnostic Interview-Revised. The results focused on the amount of radiotracer activity discovered in various regions of the test subject brains as a representative measure of microglial activation in

that subject. To their surprise, the pattern of brain region binding potential was essentially the same in the brains of control subjects as in the brains of autistic subjects; only the degree varied. In other words, in the autistic subjects the binding potential was higher, while maintaining the same general pattern of glial activation within brain regions as the age and IQ-matched healthy control subjects from the same area in Japan.

In Japan, glyphosate herbicides are widely used, including for weed control [14]. In addition, the country of Japan imports wheat from countries where glyphosate is sprayed onto wheat as a desiccant. Japan also imports rapeseed, for use as animal feed, from countries spraying glyphosate onto rapeseed as a desiccant. In the context of the theory herein advanced, these findings could fit the model of an environmental/dietary toxin.

Selected Microscopic Studies in Autistic Human Brains

Stoner et al. [15] examined post-mortem brain tissue from 22 children who died between the ages of 2 and 15. Half of the children studied had been diagnosed with autism, while the other half had not. The symptoms of those with autism varied from mild to severe. In 10 of the 11 autistic brains, they found patches of cortex in which the normal pattern of gene expression and cell organization was disrupted. These areas were only a few millimeters across (roughly a quarter to a half inch in size) across the wide expanse of otherwise histologically normal-appearing cortex.

In some of the abnormal patches, a specific layer was missing. In other patches, certain expected cells weren't present. Similar changes were found in 1 of the 11 children without an autism diagnosis, a child who had suffered from seizures. All areas of cortex sampled from autistics demonstrated such patches, however the researchers indicated that the most affected areas of the brain were the prefrontal cortex and the temporal lobe cortex, while areas such as the optical cortex were relatively spared. The researchers speculated that the defects could have resulted from the brain cells in autistics undergoing some sort of disorganization event or events during the latter part of the first trimester or the early part of the second trimester of fetal development.

This study by Stoner et al. left the researchers speculating about causation of the observed disordered patches. They mentioned that their earlier studies had demonstrated that, between the ages of 2 and 16 years, brains from autistic children are heavier than non-autistic children brains and have a relative increase in prefrontal cortex neuron numbers of up to a startling 67%. Stoner et al. also discovered that a deficit of markers of excitatory neurons was the most robust indicator of such a patch of disorganization. Furthermore, they did not believe this was a result of downregulation of genes. Rather, they speculated that the patchy areas of disorganization somehow resulted from neurons 'failing to reach their intended destination' or from de novo changes in early neurodevelopment.

Lopez-Hurtado et al. [16] conducted a microscopic study of post-mortem brains from 15 age-matched autistic and control subjects focusing on brain regions associated with the production and processing of speech. Of the brains studied, 8 were from autism patients and 7 were controls. They studied Wernicke's area (Brodmann 22, speech recognition), Broca's area (Brodmann 44, speech production) and the gyrus angularis (Brodmann 39, reading) from autistic subjects (7-44 years of age) and control subjects (8-56 years of age). These researchers found: "Striking differences in the density of glial cells, the density of neurons and the number of lipofuscin-containing neurons in the autistic group compared with the control group. The mean density of glial cells was greater in the autistic cohort than controls in area 22 ($p<0.001$), area 39 ($p<0.01$) and area 44 ($p<0.05$). The density of neurons was lesser in autism in area 22 ($p<0.01$) and area 39 ($p<0.01$). The autistic group exhibited significantly greater numbers of lipofuscin-containing cells in area 22 ($p<0.001$) and area 39 ($p<0.01$)". They concluded that "the results are consistent with accelerated neuronal death in association with gliosis and lipofuscin accumulation in autism after age seven [17]."

Selected Studies of Neuron Structure in Autism, Link to Calcium-induced Reduction in Autophagy via mTOR Hyperactivation

Tang et al. [18] were able to discern differences in post-mortem brain samples from children with autism who had died from other causes (Figure 3). Thirteen brains came from children ages 2 to 9, and thirteen brains came from children ages 13 to 20. The controls were post-mortem brains from children without autism. Tang et al. measured synapse density in a small section of tissue in each brain, counting the number of tiny spines that branch from the cortical neurons. In the control brains, spine density had dropped by about half during childhood, but by comparison in the autistic brains the density had dropped by only 16 percent during childhood.

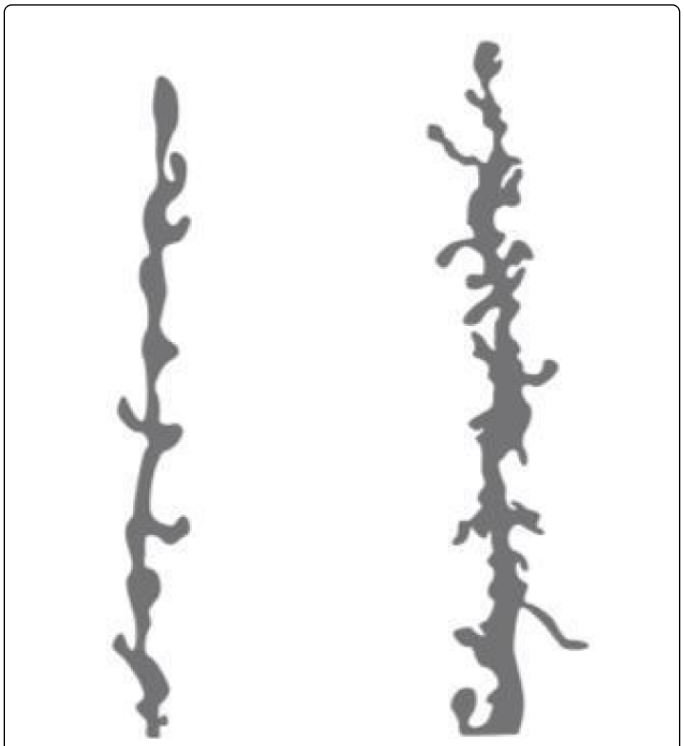

Figure 3: Modified from Tang [18]: Normal neuronal axon illustrated on left – autism neuronal axon on right with extra spines, not well 'pruned'.

The researchers then examined brains from mice, comparing autism-model mouse neurons versus non-autism normal mouse neurons. They studied Tsc2+/- ASD mice brains. These mice have alteration in the mammalian target of rapamycin (mTOR) such that the mTOR is constitutively overactive. mTOR is a serine/threonine kinase, which belongs to the phosphatidylinositol-3 kinase (PI3K) related kinase (PIKKs) family. It regulates cellular metabolism, growth, and proliferation. Tang et al.'s study of such autism-model mice with constitutively hyperactivated mTOR found signs of reduced autophagy. Autophagy is the process in neurons that, among other things, prunes neurons of excess spikes. Tang et al. [18] found that the autism-model mice, similar to the autistic children, had too numerous spikes on neurons. They deduced that this excess presence of spikes was due to a lack of adequate pruning, and postulated this was from a reduction or blockade of normal autophagy. These Tsc2+/- mice demonstrated ASD-like social behaviors.

Aberrant mTOR activation resulting in reduced autophagy is known to occur in human diseases on a genetic basis. For example, mutations in either of 2 genes (TSC1 or TSC2) have been determined to cause tuberous sclerosis complex. Among tuberous sclerosis patients, 20% to 60% are also diagnosed with autism [19,20]. It has been reported that, of all autism cases, approximately 1% to 4% are tuberous sclerosis patients [21]. Treatment of tuberous sclerosis patients with rapamycin can apparently partially correct their inherited defect of reduced autophagy.

The beneficial effect of rapamycin is thought to occur via rapamycin's action to inhibit hyperactivated mTOR and thus induce an increase in autophagy [22]. Rapamycin has been used successfully in tuberous sclerosis patients to shrink angiomyolipomas [23] and astrocytomas [24]. Clinical trials are underway to assess the feasibility and safety of administering rapalogues sirolimus or everolimus in participants with Tuberous Sclerosis Complex (TSC). The assessment (Clinical Trial NCT01929642 USA) includes measuring any reduction in autistic symptoms, such as aggressive behaviors and/or any improvements in cognitive function.

Aberrant mTOR hyperactivation is thought to occur in a wide range of ASD patients with known genetic defects [25,26], such as those autistics found to have large head size (macrocephaly) early in life [27]. This finding of large head size is reminiscent of the increased brain volume and increased CSF accumulation found in the children destined to develop autism as studied by Shen et al. [12], as discussed above. Additionally, cases of genetic disease patients linked to mTOR hyperactivation who also relatively often have a diagnosis of autism include neurofibromatosis type I, Lhermitte-Duclos syndrome, and Fragile X syndrome [21,26].

It is conceivable however, that mTOR hyperactivation can occur in autistics for which a gene sequence alteration in DNA is not present. This type of mTOR hyperactivation is theorized to occur within neurons exposed intermittently to a toxin from diet and/or environment. Such a toxin is theorized herein to alter the influx of calcium into immature neurons in a haphazard and intermittent manner resulting in reduction of autophagy in such neurons so exposed, with effects dependent on their stage of development.

Such a haphazard hyperactivation of mTOR has been reproduced experimentally and linked to the presence of amino acids. Gulati et al. [28] studied HELA cells in culture and found that mTOR activation was in relation to the level of amino acids present in the cell culture. They found that increased amino acids in the cell culture medium resulted in an increase of HELA cell intracellular calcium, resulting in increased calcium binding with calmodulin within the cell, and subsequent increased activation through the mTOR pathway. Specifically, they state in their report "We demonstrate that the rise in [Ca(2+)] (i) increases the direct binding of Ca(2+)/calmodulin (CaM) to an evolutionarily conserved motif in hVps34 that is required for lipid kinase activity and increased mTOR Complex 1 signaling". Glycine is an amino acid that is known to be present in and interact with human brain cells, in particular during neurodevelopment. A harmful glycine mimetic is herein theorized to be causative in at least some cases of autism, and to have an effect in human neurons similar to that effect of amino acids demonstrated in the cell culture studied by Guloti [28], i.e. an increased mTOR activation, thus reducing autophagy and leaving neuron spikes unpruned.

Human Post-mortem Selected Study of Autistic Brains Finds Markers of Increased Calcium in Autistic Brains versus Controls

Research on intracellular calcium in autism neurons offers a link to increased mTOR activation and reduced autophagy. Palmieri et al. [29] studied temporocortical gray matter from six matched patient-control pairs of normal and autistic brains to perform post-mortem biochemical and genetic studies of the mitochondrial aspartate/glutamate carrier (AGC). The AGC participates in the aspartate/malate reduced nicotinamide adenine dinucleotide (NAD) shuttle and is physiologically activated by calcium (Ca(2+)). AGC transport rates were significantly higher in tissue homogenates from all six autism patients, including those with no history of seizures and with normal electroencephalograms prior to death. This increase was consistently blunted by the Ca(2+) chelator ethylene glycol tetraacetic acid. Neocortical Ca(2+) levels were significantly higher in all six autism patients compared to controls, according to these researchers. Furthermore, following removal of the Ca(2+)-containing postmitochondrial supernatant, these researchers reported they then observed no subsequent difference in AGC transport rates in isolated mitochondria from patients versus controls. This result clearly demonstrates that Ca(2+) entry provoked mTOR activation, leading to reduced autophagy in association with autism.

Calcium influx relates to action at neuron membranes of glycine-related factors

An article by Avila [30] reveals the link between calcium influx into neurons and the action of ligands at membrane-embedded protein structures of neurons. For example, Avila describes the glycine receptor's (GlyR) action as follows: "GlyR activation during embryonic and early postnatal development most likely induces a depolarization of the cell membrane…which in turn may activate calcium influx".

Thus, glycine binding at the GlyR appears capable of influencing the rate of calcium influx into neurons during early neurodevelopment. As will be further explored below, we theorize action of a harmful glycine mimetic in autism causation via its influence in causing harmful and haphazard influx of calcium into immature neurons during neurodevelopment. To further clarify this theory, we identified and reviewed known facts and selected published literature regarding a candidate molecule widely dispersed in the environment and present in certain foods, namely glyphosate.

Glyphosate, candidate glycine-mimetic molecule for comparison to requirements of theory

Glyphosate, a common herbicide, is known to be produced from the amino acid glycine via addition of a phosphonomethyl group (Figure 4). In 2007, according to the US EPA, glyphosate was the most used herbicide in the United States agricultural sector, with 180 to 185 million pounds (82,000 to 84,000 tons) applied, and the second-most used in the home and garden market, where users applied 5 to 8 million pounds (2,300 to 3,600 tons); in addition, industry, commerce, and government applied 13 to 15 million pounds (5,900 to 6,800 tons).

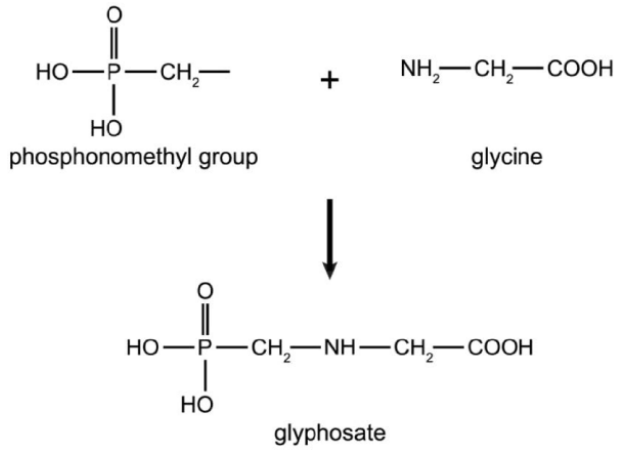

Figure 4: To glycine, chemist adds a phosphonomethyl group to make glyphosate.

Glyphosate study, glyphosate from maternal diet found concentrated in piglet fetal brain

In a study by Kruger et al. [31] of 38 malformed piglets born to sows fed glyphosate-containing feed, brain tissue samples were obtained from euthanized 1 day old piglets' brains. Following appropriate processing, these samples were tested for glyphosate using ELISA kits (Abraxis, USA). Glyphosate was found to be present in all piglet brain samples, at an average of 3.1 micrograms glyphosate per ml of sample. The authors commented that glyphosate was able to reach the fetal piglets from maternal feed, i.e. glyphosate from the feed eaten by the sow was able to pass the placental barrier. The feed, which contained 0.87 to 1.13 parts per million glyphosate, when consumed in the first 40 days of pregnancy, was associated with a rate of visible gross malformation in the piglets of 1 per 240 piglets. No microscopic evaluation of piglet brain tissue for autistic type changes was conducted.

Glyphosate usage on corn/soy crops versus rate of USA school children autism

Correlation is not proof of causation, but Swanson et al. [32] point out the near exact match in correlation between the rise of glyphosate usage on corn and soy crops in the USA, over the years 1992 to 2010, and the increase in autism rates over the same period as reported in the USA public school system (Figure 5). Among the most widely applied formulations of glyphosate is the pesticide known as Roundup®. We reviewed studies of glyphosate and Roundup® in relation to published research regarding brain tissue, including in relation to calcium influx into neurons.

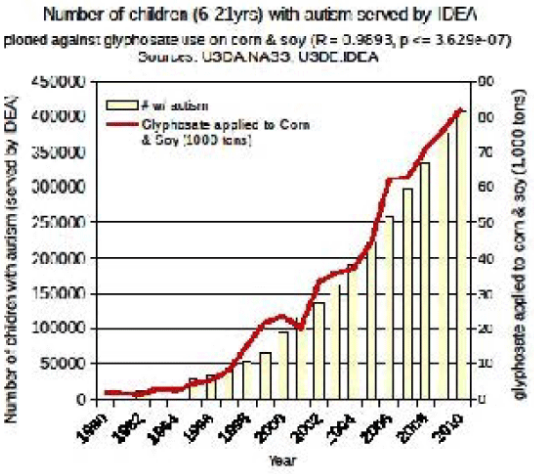

Figure 5: From Swanson et al. [32], used by permission: Near exact match of tons of glyphosate applied to corn/soy versus number of children with autism as served under IDEA (US Department of Education, Individuals with Disabilities Education Act).

Glycine chelates, Glyphosate, manganese and the glutamate-glutamine cycle

Glycine, the simplest amino acid, has a molecular weight of 75, and naturally forms chelates with cations. The strength of glycine chelates is ideal for biologic processes, for example aiding mineral absorption from the intestine, while not overly avidly binding minerals which are needed for use in the body, such as for enzymatic reactions. When discussing a putative harmful glycine mimetic in the brain, it is useful to consider chelation in regard to the Glutamate-Glutamine cycle, and its apparent dysfunction in autism.

We begin with the synapse, the space between two neurons where chemical signals pass. Receiving the signal are receptors at the post-synaptic neuron membrane. Among these receptors are the NMDA receptors, which are neural membrane-embedded protein structures. When activated, the NMDA receptor opens to allow calcium to enter the neuron. The activation process, as will be discussed in detail below, includes the simultaneous binding to the NMDA receptor of both glutamate and glycine or a glycine mimetic [33].

Studies of autism and glutamate have documented that, in autism patients, glutamate activity is impaired [34], both in regard to actions at the synapse [35] where glutamate is released, and in the resultant calcium signaling, as it relates, for example, to dendritic growth [36]. A study by Page et al. [37] demonstrated that the ASD patients studied had a significantly higher concentration of glutamate in the amygdala-hippocampal region of the brain than did normal controls. These findings suggest the Glutamate-Glutamine cycle is disrupted in autism.

The cycle can be viewed as starting with the step where glutamate is released into the synapse by the pre-synaptic neuron. The released glutamate can participate in binding the NMDA receptors of the post-synaptic neuron, thus transferring a signal. Such NMDA receptor activation is subject to other factors, as will be discussed below, but if

the activation leads to excess calcium inflow into neurons, this can be problematic. The Glutamate-Glutamine cycle can be viewed as one of the natural controls to prevent such excess calcium inflow into neurons.

As the next step of the cycle, the free glutamate within the synapse space is normally quickly taken up for recycling by astrocytes. This removal of glutamate from the synapse helps prevent over-activation of the NMDA receptors. Within the astrocytes, the enzyme glutamine synthetase (GS) normally converts glutamate to glutamine. Glutamine is then returned from the astrocytes to the neuron. Within the pre-synaptic neuron, glutamine is converted back to glutamate for storage in internal vesicles. These vesicles will later move to the pre-synaptic neuron membrane and subsequently this glutamate will be released back into the synapse, where the cycle repeats [38].

In essence, GS can be viewed as helping protect neurons from excess glutamate activity/toxicity. However, GS activity depends on manganese as a cofactor, and this is where a putative harmful glycine mimetic could interfere. Glyphosate, as the candidate glycine mimetic, has a well-known action as an exceptionally avid chelator of cations, including manganese. Thus, glyphosate is viewed by some experts as having the potential to interfere with the free supply of manganese within astrocytes for use by GS within the Glutamate-Glutamine cycle [39]. This is theorized as one mechanism by which a putative harmful glycine mimetic, operating locally in patchy areas of the brain, might cause damage to neurons by increasing glutamate to toxic levels within the synapse, thus spiking calcium inflow haphazardly into neurons. This theory appears to correlate to the findings of Page et al. [37] regarding excess glutamate in autistic brains in the amygdala-hippocampal area and the findings of Stoner et al. [15] regarding patchy areas of cortical disorganization found in brains of autistic patients.

As an example of its avidity in binding manganese and magnesium, glyphosate has been shown to deplete manganese and magnesium levels in young leaves of non-transgenic soybean plants exposed to glyphosate [40]. A recent study on dairy cows fed GMO Roundup-Ready feed showed dramatically reduced levels of serum manganese in association with glyphosate residues in the urine [41]. Autism has been linked to reduced manganese levels in the baby teeth of autistics [42]. Seizures, which have been associated with low serum manganese [43,44], are more prevalent among children with autism [45]. We will further explore these matters and related factors below.

Round-up's effects on animal brain slices - calcium influx, apparently via NMDA receptor activation

Excess calcium entry into neurons is a well-known pathway to neuronal damage and/or disruption of neurodevelopment. In the study conducted by Hyrc et al. [46], increasing concentrations of calcium were applied to in vitro cultures of disassociated embryonic neurons from 15 to 18 day old mice embryos. Such concentrations of calcium were found to be predictive of neuronal death during NMDA stimulation. Cattani et al. [47] studied the effect of glyphosate on immature neurons of the hippocampus in rats. Maternal rat exposure to the pesticide involved treating dams orally with 1% Roundup® (0.38% glyphosate) during pregnancy and lactation (until 15 days old). Hippocampal slices from 15-day-old rats were acutely exposed to Roundup(® (0.00005-0.1%) during 30 min, and experiments were carried out to determine whether glyphosate affects (45)Ca(2+) influx and cell viability. In this preparation, these researchers investigated the pesticide's effects on oxidative stress parameters, (14)C-α-methyl-amino-isobutyric acid ((14)C-MeAIB) accumulation, as well as glutamate uptake, release and metabolism. They found that acute exposure to Roundup(® (30 min) increases (45)Ca(2+) influx (apparently by activating NMDA receptors and voltage-dependent Ca(2+) channels), leading to oxidative stress and neuronal cell death. Signal transduction pathways involved in the Roundup®-induced $^{45}Ca_2^+$ uptake showed that either AP5 (a NMDA receptor antagonist) or KN-93 (a Ca2+/calmodulin-dependent protein kinase II selective inhibitor) prevented Roundup®-induced 45Ca2+ influx.

Cattani et al. [47] also found that such acute exposure of animal brain slices to glyphosate increased (3)H-glutamate release into the synaptic cleft, decreased glutathione (GSH) content and increased the lipoperoxidation, characterizing excitotoxicity and oxidative damage. They also observed that both acute and chronic exposure to Roundup(® decreased (3)H-glutamate uptake and metabolism, while inducing (45)Ca(2+) uptake and (14)C-MeAIB accumulation in immature rat hippocampus.

In view of the above suggestion that glyphosate has interaction at neural membrane-embedded receptors, a review was carried out regarding structure and function of neural membrane-embedded proteins, particularly as they relate to glycine, calcium influx and potential sites for interaction of glyphosate and/or glycine mimetics.

Neurotransmitters and membrane-embedded protein structures in neurons

Glycine is widely recognized as a neurotransmitter, i.e. glycine can, under specific circumstances, have an effect on the flow of ions across a neuron's membrane. The neuron membrane is a lipid bilayer with embedded proteins. Some of these embedded proteins are pumps, such as the sodium-potassium ATPase pump, while many embedded proteins are ion channels. These ion channels are transmembrane proteins with a complex configuration that includes a pore through which ions can pass. Some channels permit passage of ions via opening of the pore as a result of membrane depolarization, and/or by the binding to the membrane-embedded protein of certain chemical ligands. Most channels have selectivity; i.e. when open they allow only certain ions to pass.

Neural membrane-embedded proteins/receptors: Sites for putative harmful glycine-mimetic action

Glycine receptor (GlyR)

GlyRs are formed by the assembly of five subunits which arrange symmetrically around a central pore. The beta subunit is the only one which interacts with the anchoring protein gephyrin, making it key to anchoring GlyRs at the area of the synapse. Of the alpha units, four types are known. Action of the GlyR, such as in terms of speed of opening kinetics, depends on which alpha units are present within a particular GlyR. While the alpha 1 beta combination displays the fastest kinetics, alpha 2 containing receptors are most abundant during neurodevelopment.

GlyR's can be activated by glycine or by taurine or alanine. When open, chloride can traverse the pore, and flow is according to chloride gradient. In regard to autism, mutations in genes encoding the alpha 2 subunit have been found in patients who carry a diagnosis of autism [48].

The excellent article by Avila [30] reviews known facts about glycine receptor activity during neurodevelopment. The brain's early cortical development includes formation of mini-columns via migrating neurons [49]. Interneurons make up only about 15% of neurons, but are important to establishment of these first brain circuits [50,51]. GlyRs have been demonstrated to have a role in controlling cortical interneuron development [30] and migration.

A disruption of migration of neurons can thus be theorized to be induced when GlyR activation is disrupted. In fact, abnormal halting of neuron migration can be demonstrated by unopposed action in application of glycine to neurons in vitro [30]. The findings of Stoner et al. of patches of disordered clusters of cells in brains of autistics suggests migration of neurons is abnormal in autistics. GlyR malfunction as a result of action of a harmful glycine mimetic is herein theorized as causative of autism in at least some cases.

Figure 6 illustrates the GlyR ligand binding site. As mentioned, research has demonstrated that besides glycine, several other molecules can bind to the GlyR and alter the activation of GlyR. For example, strychnine is known to be an antagonist of the GlyR, as is caffeine. GlyR activation has an effect on clustering of receptors at post-synaptic sites [17].

A disruption of GlyR activation theoretically induces alterations in chloride outflow for immature neurons, and thus empowers haphazard disruptions for calcium inflow into immature neurons via the NMDA receptor channel (subject to occurrence of other coincident actions at the NMDA receptor as will be discussed below). This putative harmful glycine mimetic's action at the GlyR, is envisioned to be via an exposure(s) from diet and/or environment, dosing the fetus or child in an intermittent variable-dose manner. The resulting haphazard calcium influxes theoretically enable a spectrum of severity in disrupted neuron migration, reflective of the spectrum of symptom severity in ASD cases clinically.

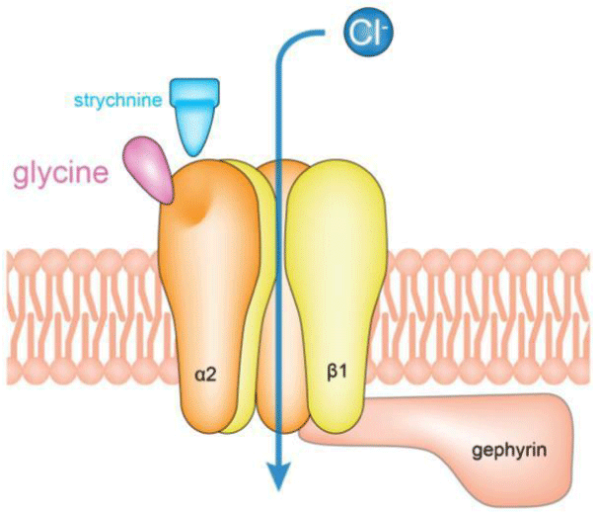

Figure 6: Glycine receptor (GlyR) viewed with 5th subunit (an alpha) removed.

The mechanism by which outflow of chloride from an immature neuron affects calcium inflow into that immature neuron, has to do with the effect of chloride outflow on the electrical charge at the neuron's membrane. A neural membrane usually retains a negative charge within the cell, and a positive charge on the exterior. However, at times, such as when chloride outflow through the GlyR is high, the charge of the membrane can change, leading to what is known as membrane depolarization. Membrane depolarization can then alter activity at other membrane-embedded protein structures, such as at the NMDA receptor. Because like charges repel each other, a depolarization of the membrane, which produces a temporary positive charge nearer the NMDA receptor, can dislodge the positively charged magnesium ion from its blocking position within the NMDA receptor, as illustrated in Figure 7.

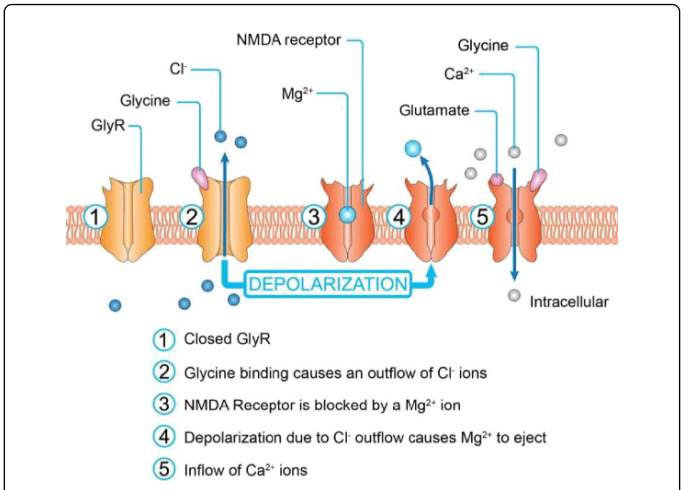

Figure 7: NMDA receptor, note the magnesium ion is dislodged from within the NMDA channel by the depolarization caused by chloride outflow at the GlyR when glycine binds there.

However, the NMDA receptor channel will not open to calcium inflow just because the magnesium ion exits the channel of the NMDA receptor. The two binding sites of the NMDA receptor must also be properly filled. A molecule capable of agonist binding at the so-called glycine binding site of the NMDA receptor must be in place. Also, a molecule of glutamate must be bound at the glutamate binding site of the NMDA receptor. Only when these three events occur at the same time, i.e. the magnesium ion's exit from the NMDA channel, the binding of an activating molecule to the glycine site of the NMDA receptor, and the binding of glutamate to the glutamate site of the NMDA receptor, only then will the NMDA channel allow calcium to flow into the neuron.

From this complex set of requirements it is clear how carefully neurodevelopment guards against haphazard or excess entry of calcium into immature neurons. Conversely, as theorized herein, when a harmful glycine mimetic is able to disrupt this carefully choreographed system, the normal neurodevelopment sequence does not occur. Recall how calcium excess within a mouse brain model of autism was associated with reduced autophagy and malformed poorly 'pruned' neurons. It seems that proper neurodevelopment is critically dependent on the correct and beneficial control of calcium entry into immature neurons.

Taurine, an amino acid, is also capable of binding the GlyR. When taurine binds to the GlyR, such taurine binding, like the binding of glycine, opens the GlyR ion channel to gradient directed flow of chloride. However, taurine binding appears to induce only

approximately half as much chloride flow as compared to glycine binding. In effect, taurine is a glycine mimetic which reduces chloride flow compared to glycine binding of the GlyR. This implies a reduced opportunity of chloride flow to spur depolarization of the immature neuron's membrane. Thus, taurine is seen as a partial agonist of the GlyR.

Taurine tends to modulate the action of glycine at the GlyR, i.e. taurine's presence can serve as a natural competition with glycine. This natural competition results in fewer membrane depolarization events. In effect, taurine's binding at the GlyR during neurodevelopment is a partial interference with glycine's ability to boost calcium inflow events for the neuron. This has profound potential to influence neuron migration during neurodevelopment.

Because taurine, as mentioned above, only permits approximately half the chloride flow at the GlyR when compared to glycine binding the GlyR, it is thus less likely that a membrane depolarization will occur with taurine bound to the GlyR (Figure 8). In fact, the amino acid taurine is reported [52-54] to serve to counteract glutamate-induced elevations in intracellular calcium ions within neurons, and thus taurine confers protection against neurodegeneration. The concentration of taurine compared to the concentration of glycine in the vicinity of the GlyR results in a choreographed competition. Taurine, in theory, will likely similarly compete for binding versus glyphosate, and therefore taurine likely would tend to protect from any theorized harm from glyphosate binding at the GlyR.

Studies by Leon et al. [54] demonstrated a neuron-protective effect for taurine thought to operate via modulating the calcium influx to neurons which otherwise would occur due to glutamate at the NMDA receptor. Glutamate applied without taurine was able to drive apoptosis (neuron cell death) via release of cytochrome C. Taurine application along with glutamate was shown to prevent such apoptosis.

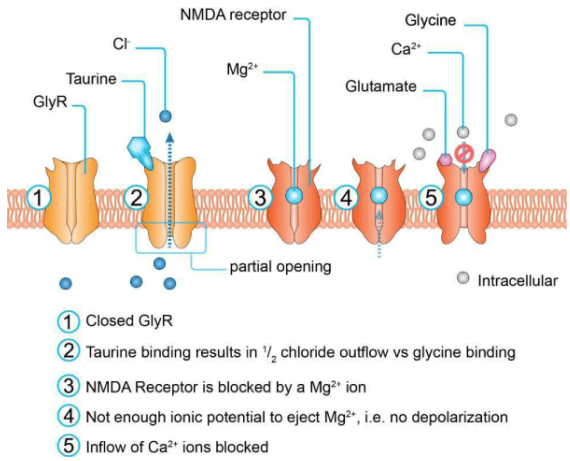

Figure 8: Taurine binding to GlyR reduces chloride flow by approximately 50% compared to flow from glycine binding of GlyR, so no depolarization occurs with taurine bound to GlyR, thus magnesium ion remains within NMDA receptor channel, continuing to block calcium from entering immature neuron.

The level of taurine present in the brain is known to progressively increase during embryogenesis. At the same time, the different GlyR assemblies, from incorporation of different alpha subunits, means that another factor is present beyond taurine concentration. Different GlyRs vary in their sensitivity to taurine. Homomeric GlyRa2, for example, is 10 times less sensitive to taurine [30] compared to the most sensitive GlyR.

Thus the choreography of neurodevelopment is tuned to genetic distribution of different membrane-embedded receptors/proteins over time, and to effects of local concentrations of competing receptor agonists. In regard to the theory herein advanced, we theorize the harmful action of a putative glycine mimetic acting at the GlyR to disrupt such delicate choreography. This damaging effect on neurodevelopment is notably a local and time-sensitive effect, rather than dependent solely on overall dose of toxin to the fetus or mother.

Another balanced mechanism during neurodevelopment by which neurons and axons perform coordinated migration is the frequency of spontaneous waves within a neuronal circuit, i.e. waves of depolarizing or 'bursting' activity. The effects within the brain of a disruptive decrease of the local frequency of such spontaneous bursting activity via toxins that act at the GlyR was demonstrated by Hansen et al. [55,56].

They examined white leghorn chick embryos' spinal neuron migration in ovo by cutting a square hole through the egg shell and placing a pipette via which they administered GlyR antagonists, strychnine or picrotoxin. By thereby decreasing calcium transient influxes locally, and thus decreasing the normal spontaneous rhythmic bursting activity (RBA) of developing neuron circuits, they noted migration abnormalities. Such GlyR antagonists, when administered at certain days during gestation, also altered gene expression locally of genes which normally code for neuron axon guidance/adhesion proteins.

Glycine as ligand, binding to NMDA receptor

As discussed above, the NMDA receptor has a binding site for both glutamate and glycine (D-serine also can bind the glycine site of the NMDA receptor). Only when both of these binding sites of the NMDA receptor, the glutamate site and the glycine site, are agonistically filled, and only if the magnesium ion is simultaneously displaced from the NMDA channel, only then will the NMDA channel allow calcium to enter the neuron. As mentioned, this tight control of calcium entry has import for neurodevelopment. The amount of calcium entering via the neuron's NMDA receptors is known to be further modulated by pH and zinc.

If a molecule of the herein theorized putative harmful glycine mimetic binds more avidly than glycine at the glycine binding site of the NMDA receptor, then the chances of calcium inflow into immature neurons could theoretically be increased. This is because the theorized more avid binding of the putative harmful glycine mimetic to the NMDA receptor's glycine site, would extend the time for the simultaneous occurrence of the other two events, i.e. for the simultaneous exit of the magnesium ion from the NMDA channel and the simultaneous binding of glutamate to the NMDA receptor's glutamate binding site.

Glycine concentration at synapse, glycine transporter protein T1 (GlyT1)

Another mechanism by which a spectrum of severity of autism is envisioned to occur, from the action of the putative harmful glycine mimetic, relates to glycine transport proteins. Neurotransmitter glycine participates in a recycling mechanism of uptake of glycine from

the synapse by cells in the brain. Storage of glycine in the pre-synaptic neurons occurs for a time, followed by re-release of the stored neurotransmitter back into the synapse (Figure 9).

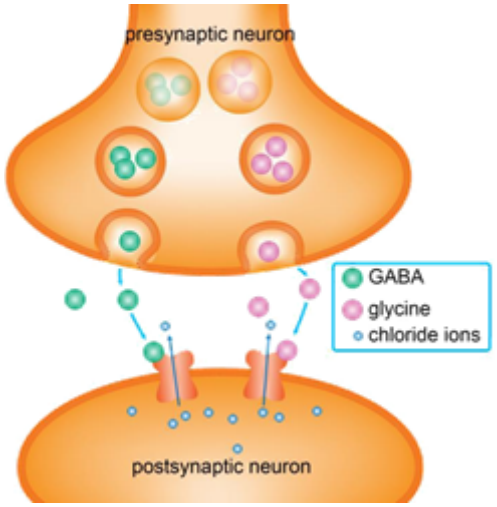

Figure 9: Neurotransmitter glycine is taken up from synapse by GlyT1 transporter protein (not shown), stored, then re-released into synapse to bind glycine receptor of post-synaptic neuron.

Glycine transporter proteins GlyT1 and GlyT2 are membrane-embedded proteins specific for glycine handling. The primary role of GlyT1 is thought to be to maintain glycine concentrations below saturation level at postsynaptic NMDA receptors, thus modulating influx of calcium into neurons. GlyT1 is known to be subject to inhibition by glycine mimetics. For example, sarcosine, one of the naturally occurring N-methyl analogues of glycine, is a known inhibitor of GlyT1. Sarcosine is also one of the possible metabolic breakdown products of glyphosate.

We theorize that glyphosate directly, or sarcosine from glyphosate breakdown, act via GlyT1 inhibition to locally and intermittently inhibit the uptake of glycine from the synapse. We theorize that, during neurodevelopment, such inhibition of GlyT1 permits excess synaptic glycine to accumulate. Thus glycine could bind to saturation the NMDA receptor of an immature neuron, thus increasing calcium entry via the NMDA receptors into the immature neuron, damaging neurodevelopment.

In regard to GlyT1 inhibition and neurodevelopment, the work of Schmitz et al. [57] is of interest. They performed unilateral intrastriatal injection in mice of toxin 6-hydroxydopamine and studied the re-enervation response with and without GlyT1 inhibition. In the studied mice whose glycine transporter protein1 function was inhibited pharmacologically for 4 weeks, the axon sprouting of the neurons responding to the denervation was, at 7 weeks, twice as dense as controls. This 'over-sprouting' occurred via action of the NMDA receptors, according to Schmitz et al.

If a re-enervation sequence can be conceptualized as similar to the original enervation sequences of neurodevelopment, then the study of Schmitz et al. might provide a relevant theoretical explanation. A spike of putative harmful glycine-mimetic molecules in a localized area of cortex in a developing human fetal brain might produce a disruptive effusion of neuron spikes due to local blockade of GlyT1 by the harmful glycine mimetic (or its metabolite). Such patches of putative over-exuberant neuron spikes are reminiscent of the findings of Tang et al. [18], and reminiscent of the increased neuron density found in patches in brains of autistic children by Stoner et al. [15].

In fascinating studies [56,58] by Hansen et al., these researchers administered the GlyT1 inhibitor sarcosine via in ovo method during neurodevelopment of white leghorn chick embryos. They documented that sarcosine caused an increase in the frequency of calcium transients, which translated into an increase of the frequency of spontaneous rhythmic neuronal bursting activity. They found this produced abnormalities in neuron/axon migration.

In summary, Hansen et al. confirmed that rhythmic bursting activity (RBA) of neuron circuits is key for early pathfinding decisions by neurons. Moderate slowing of the frequency of RBA causes "motoneurons to make dorsoventral (D-V) pathfinding errors and to alter the expression of molecules involved in that decision." Conversely, moderate speeding up of RBA in neuron circuits strongly perturbs "the anteroposterior (A-P) pathfinding process by which motoneurons fasciculate into pool-specific fascicles at the limb base and then selectively grow to muscle targets." In their studies, these researchers found that resumption of normal frequency of RBA sometimes allowed axons to correct the A-P pathfinding errors, perhaps leaving aberrant nerves.

Aberrant connectivity has, in fact, been documented in the brains of autistic children [59] using resting state functional magnetic resonance (fMRI) imaging. Di Martino et al., studying autistic children using fMRI methods, concluded that their examination of functional connectivity (FC) of striatal networks in children with ASD revealed "abnormalities in circuits involving early developing areas, such as the brainstem and insula, with a pattern of increased FC in ectopic circuits that likely reflects developmental derangement rather than immaturity of functional circuits." This pattern also fits our theory of a dietary/environmental sourced toxin acting locally as a harmful glycine mimetic, having patchy effects in the CNS beyond the cortex.

Neuron migration has been visualized. Avila et al. [60] demonstrated in vivo, using cultured brain slices from mice embryos, that actomyosin contractions alter neuron migration. These researchers recorded an example of neuron migration in video format and have made the recording available for viewing online as movie S2 of their open access report [60].

In summary, this delicately choreographed neurodevelopmental process in humans, including the precise frequency of waves of depolarization in synchronized neurons within circuits, this pattern of neurons which alternately sprout, prune and migrate, this choreography can be locally disrupted, we theorize, by a relatively small number of molecules of a harmful glycine mimetic. From the available research literature, such disruption, for example via the GlyR and/or the GlyT1 and/or the NMDA receptor, can reasonably be theorized to produce neuronal/axonal migration defects reminiscent of those found in studies of brains of autistic patients.

Next, we will address the neuron membrane-embedded protein structures known as Na-K-Cl cotransporter 1 (NKCC1) and K-Cl cotransporter 2 (KCC2).

Glycine, NKCC1 versus KCC2 in neurons, brain weight

Within the brain during normal neurodevelopment, the types and numbers of membrane-embedded proteins will change as neurons

mature. As illustrated in Figure 10, in immature neurons the membrane will typically have more NKCC1 proteins embedded, and fewer KCC2 proteins. One result is that immature neurons tend to keep internal chloride concentration higher.

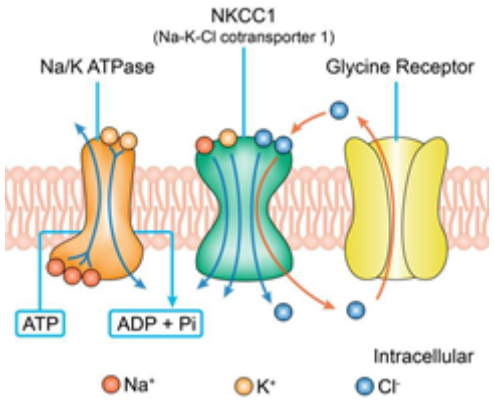

Figure 10: In normal circumstances, the membranes of immature neurons include numerous NKCC1, fewer KCC2; intracellular chloride concentration tends to be higher in normal immature neurons as compared to mature neurons.

As neurons proceed through normal fetal development, they will begin to manufacture fewer of the NKCC1 proteins and instead manufacture more of the KCC2 proteins. Therefore, mature neurons will have more KCC2 proteins embedded in their membrane and fewer NKCC1 proteins embedded, as illustrated in Figure 11. The result, in normal circumstances, is that mature neurons tend to have a lower concentration of chloride internally.

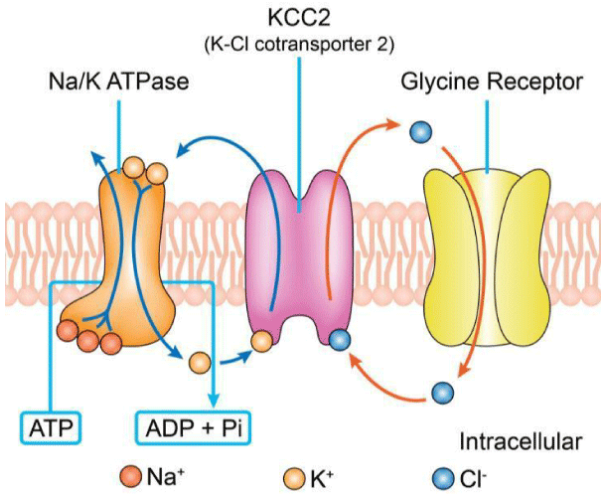

Figure 11: In normal circumstances, mature neurons have fewer NKCC1 and more KCC2; intracellular chloride concentration tends to be lower in normal mature neurons as compared to immature neurons.

In both the immature neuron and the mature neuron, the glycine receptors (GlyR) aid in keeping the internal chloride concentration within expected bounds (Figure 12). The programmed normal change of neuron membrane-embedded proteins predominance, from predominately NKCC1 (immature neurons) to predominately KCC2 (mature neurons), is commonly referred to as the 'GABA switch.' This reflects the fact that gamma amino butyric acid (GABA), in normal circumstances, acts to inhibit hyperactivity in mature neurons, such as during birth and post-natally [61]. The GABA switch is often found to be impaired in autism patients, a finding that we theorize might be related to action of a harmful glycine mimetic during neurodevelopment, as further described below.

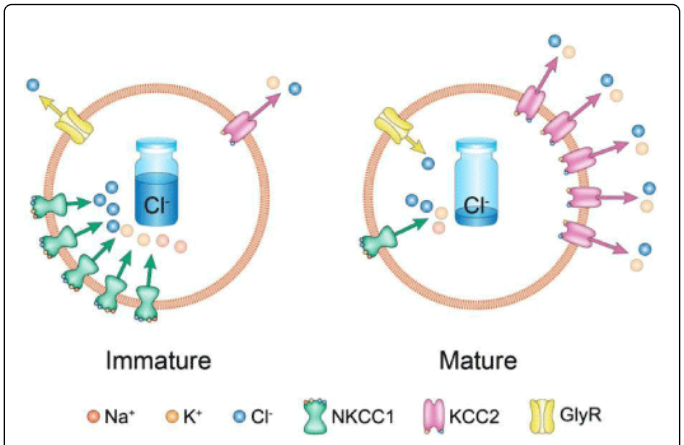

Figure 12: Under normal circumstances, chloride flows out of an immature neuron when GlyR is open. Under normal circumstances, chloride flows into a mature neuron when GlyR is open.

Interestingly, a partial agonist of the GlyR is caffeine. It is noteworthy that autistic children receiving a low dose of daily caffeine over weeks or months have been reported [62] to sometimes experience improvement of autism symptoms as a result of the caffeine.

As previously mentioned, it is well known that the brains of autistic children, in their early childhood years, are heavier than controls. We postulate that this weight increase is caused by increased water content of the brain, such as in neuropil, glial cells and/or neurons. We believe such water retention could be a response to excess cell-internal chloride, caused by impaired chloride exit following glyphosate binding to glycine receptors. Chloride retention has been implicated in the brain swelling associated with edema following brain trauma [63]. Furthermore, sarcosine, a breakdown product of glyphosate, has been shown to activate glycine receptors, but with a reduced effect on the chloride channel [64]. Hence, chloride can be expected to accumulate over time within the cell due to reduced efflux in response to sarcosine or glyphosate binding.

Glycine and the choroid plexus

With regard to cerebrospinal fluid (CSF), production is typically balanced to resorption. The average adult produces approximately 500 mL of CSF per day, but because CSF is constantly resorbed, only 100-160 mL is present in adults at any one time under normal circumstances. It has long been thought that CSF returns to the vascular system by entering the venous sinuses of the dura via the arachnoid granulations (also known as villi). However, recent research [65] has suggested that CSF flow along the cranial nerves and spinal nerve roots allows at least some CSF to flow into the brain's lymphatic

channels. Such flow may play a substantial role in CSF resorption, in particular in the neonate or fetus, in which arachnoid granulations are sparsely distributed.

This apparent interplay between CSF and lymphatic fluid opens the door to new discoveries in brain metabolism and immune activity. For example, the possibility of harmful/reactive substances entering the CSF from lymph might be considered. Lymph is the fluid that bathes lymph nodes, interacts with thymus-derived cells [66] and flows throughout the body using lymph channels. Lymph re-enters the blood via the lymphatic duct in the chest. These factors may be explored in future studies to explain the immune alterations in autism.

With regard to the present theory, the precise details of glycine's action with respect to the choroid plexus are, as yet, not well studied or understood. Nevertheless, the excess CSF accumulation visible on the MRI images reported by Shen et al. [12] in children destined to develop autism is perhaps tentatively linkable to glycine, and/or a theorized harmful glycine mimetic.

For example, presence of a glycine receptor site in sheep choroid plexus was established by Preston [67]. A co-transporter of GABA and glycine known as GAT-2 has been reported within human choroid plexus cells. Studies by Schlessinger et al. [68] on the function of the GAT-2 transporter have revealed that, in addition to transporting GABA and glycine, the GAT-2 transporter also has a strong interaction with a glycine mimetic known as glycylglycine. Thus, it is theorized herein that a harmful glycine mimetic could act via glycine sites in the choroid plexus to increase CSF production. The details of such action are, as yet, unclear.

Relevant Treatment results in ASD patients

In order to further evaluate the theory herein advanced, selected pharmacologic treatment successes in autism are considered in light of neural membrane-embedded proteins and neurotransmitters.

Calcium channel blockers/modulators, verapamil and ketamine

In reviewing the literature regarding calcium channel blocker use in treatment of autism, no randomized clinical trials were found. The authors of the present article were struck, however, by an online blog series of reports by the non-scientist father of an autistic son. We hesitate to mention this anecdote here, but feel that even anecdotal evidence can sometimes point the way to clues of causation. Therefore, we present the story of an autistic boy, Anthony (not his real name). The father's blog post (at Epiphanyasd.blogspot.com) indicates that during one summer, Anthony was dealing with allergies and was struggling with 'aggressive' symptoms of autism, as per his father's reports online. As the blog describes, Anthony was saying 'Be nice' and 'to hit your head' associated with attempts to self-control his flares of aggressive autistic symptoms.

The father reports giving Anthony an anti-histamine during that summer, but found it was only effective for Anthony for a couple of hours per dose. The father, as he mentioned in his posts, had researched the work of Italian professor and clinician Antonio Persico [69] in regards to calcium and the autistic brain. The father reports he researched the calcium channel blocker verapamil. Satisfied that a low dose of verapamil would be safe for his son, and seeing Anthony continuing to demonstrate aggressive symptoms, the father began Anthony on a dose of 20 milligrams verapamil. The father recorded online the circumstances regarding his son as follows: "One afternoon, I decided to give a very small dose (20 mg) of Verapamil, and before my eyes, the anger and agitation began to fade and was replaced by calm. It was the most amazing experiment that I have witnessed and within 20 minutes there was complete calm".

While anecdotal reports clearly have obvious limits, they may sometimes serve as a clue to areas deserving of further study under application of a validated scientific method. In addition, although the authors of the present article do not recommend that parents independently apply non-prescribed medical regimens for their autistic children, it is heartening to read the follow-up note in the blog concerning Anthony. As the father described it: "In the following weeks, I would still hear Anthony say `be nice,' but this was no longer followed by any aggressive behavior. The trigger was still there to energize these channels, but they had been blocked by Verapamil. It was like firing a gun, but with no ammunition; there was a `click,' but no `bang.' "

Another calcium channel blocker, ketamine, has been reported in the scientific literature to have value in treating autism. Ketamine is a known antagonist of the NMDA receptor, capable of reducing the influx of calcium into neurons. Successful anesthesia of 5 autistic children has been reported by an anesthesiology group using oral ketamine preoperatively [70]. The anesthesiology group indicated that they found the symptoms of autism were less likely to interfere with the surgery if the patient was given ketamine orally in the preoperative period. This report of ketamine success in reducing symptoms of autism appears to link reduction of calcium entry into neurons with reduction in autism symptoms.

NKCC1 Chloride channel and bumetanide

In the brain, the diuretic bumetanide blocks action of the membrane embedded protein NKCC1, and thus can alter internal chloride concentration within neurons. Bumetanide, when given to pregnant mice with models of autism, has been reported to prevent or reduce autistic behavior in offspring [61]. In humans, Lemonnier et al. [71] reported successful use of bumetanide in a 10 month treatment study in adolescents/young adults with autism. These researchers stated that their treatment was based on their understanding that bumetanide decreases the level of intra-neuronal chloride (Cl-)i and reinforces the natural GABA inhibition of excitation of neurons in autistic patients. They report reduced severity of autism symptoms and indicate that bumetanide treatment improves emotion recognition and enhances the activation of brain regions involved in social and emotional perception during the perception of emotional faces by bumetanide-treated autistics.

The theory herein advanced proposes the harmful action of a putative glycine mimetic to over-concentrate chloride within immature neurons. Bumetanide, by blocking such chloride-concentrating action, might at least temporarily relieve such excess chloride concentrations within immature neurons.

Glutamate and the Multi-system nature of autism, food choices linked to putative harmful glycine-mimetic

Certain chemistry findings are typical of autistic patients, such as elevated plasma and brain glutamate [72] and an altered amino acid profile in the blood [73]. In fact, autism is widely recognized to be a multi-system disorder [74]. For example, pediatricians have described

in autistics a somewhat characteristic pattern of signs and symptoms including smelly bowel movements, bloated bellies, dry skin and frequent colds and ear infections. Such multi-system manifestations in autism will be very briefly addressed below to indicate their linkage, within the theory herein advanced, to a putative causative harmful glycine mimetic.

Modern processed food is well known to contain excess ammonia due to the methods of food processing. For example, as far back as 1973, elevated ammonia content of gelatin, cheese, breakfast cereal, bacon, corn, peas, numerous other vegetables, cheddar cheese, buttermilk and other foods was reported in the scientific literature [75]. Injecting ammonia into meat as a means to control E. coli has been accepted practice for years, though only recently widely publicized. Part of the reason the public was not generally aware of the elevated ammonia content of hamburger and what is known as 'pink slime' as a meat component, was that the amount of ammonia used was not required to be listed as an ingredient on the label due to the fact that the government viewed the ammonia as a processing chemical.

Under normal circumstances, excess ammonia would be a burden for normal human digestion. We theorize this burden is made more difficult by the presence of a harmful glycine mimetic within such high ammonia food. We theorize that the combination in food of excess ammonia and a harmful glycine mimetic having biocide capability alters the gut microbiome, the gut lining, the blood chemistry, and has implications for neurodevelopment.

In order to better describe this theory, it is instructive to begin by reviewing the findings of a study of gobie fish by Peh et al. [76]. They studied ammonia detoxification in the gobie fish euryhaline Bostrychus sinensis exposed to excess ammonia in a hyperosmotic environment, whereby drinking was essential for osmoregulation. They found alterations in intestine/contents, which alterations they linked to action of the enzyme system the fish uses to de-toxify ammonia. This enzyme system is the well-known glutamate dehydrogenase (GDH) - glutamine synthetase (GS) system. The expected products of the GDH-GS system in detoxifying ammonia include glutamine and glutamate, with perhaps un-detoxified ammonia left over if the ammonia substrate was in excess.

In their study of the gobie fish, Peh et al. did find in intestine/contents a significantly elevated glutamine level after ammonia exposure, a sign the GDH-GS system was in operation and detoxifying ammonia. But they did not find elevated glutamate in the intestine/contents. They reasoned that the glutamate must have been generated from the GDH-GS system, but had apparently been absorbed into the blood stream of the fish, likely so that this relatively toxic glutamate could be either detoxified within other organs, or changed into other amino acids.

Turning now to studies in autistics, Aldred et al. [73] studied blood samples from patients with autism or Asperger syndrome and blood samples from their siblings and parents. They found that all family members had the same pattern, with raised glutamate levels in their plasma, but reduced plasma glutamine. This is reminiscent of the pattern of glutamine in the intestine and glutamate absorbed into the circulation from ammonia detoxification as found by Peh et al. in the gobie fish. This pattern in the autism families studied by Aldred et al., we believe, is due to the detoxification of excess dietary ammonia by their human gut microbiome.

But, autistics it seems, also have other markers suggesting detoxification of excess food ammonia. In addition to the decreased plasma glutamine and increased plasma glutamate, Aldred et al. also measured plasma levels of other amino acids. They found autistic patients and their family members had elevated plasma levels of alanine, phenylalanine, asparagine, tyrosine and lysine when compared to controls.

These findings suggest to us that autistics and their family members were eating, generally speaking, a very similar diet of high-ammonia foods as compared to controls, food that also putatively contained a harmful glycine mimetic. This pattern of plasma amino acid elevations in autistics and their family members, we believe, fits the pattern of excess food ammonia with detoxification via GDH-GS. Our view is that some of the excess glutamate absorbed from the intestine of the autistics and the intestine of their family members became substrate. We believe, for example, some glutamate was transaminated by alanine aminotransferase into lactate-derived pyruvate to form alanine. Elevated plasma alanine was found by Aldred et al. [73] in autistics and their family members.

Autism, gut microbiota

Damage to neurodevelopment within the fetus is herein theorized from maternal exposure to a putative harmful glycine mimetic. However, neurodevelopment continues after birth. This raises the possibility of damage to developing neurons occurring from a harmful glycine mimetic in the child after birth. For example, once the newborn begins developing his or her own gut microflora, exposure to a harmful glycine mimetic with biocide capabilities could alter gut microflora, contributing to excess glutamate in blood and in the brains of autistics if excess ammonia is present. Gut flora alterations have been well documented in autism [77-79], as well as leaky gut syndrome [80,81], excess fecal content of short chain fatty acids and elevated ammonia [82]. In vivo studies on rats have shown that ammonia administered via injection of ammonium acetate activates NMDA receptors in the brain, leading to increased intracellular calcium, calcium uptake into mitochondria, and initiation of neuronal death [83].

Regarding gut microbiota, the excellent review and analysis by Krajmalnik-Brown et al. (65) found a 'hyper-Westernization' of the gut microbiota of children with ASD. They speculated this alteration "could indicate that gut microbiota differences that are driven by unique aspects of the Western lifestyle compared to the developing world lead to the association of unique gut microbiota composition with ASD." If indeed it is present, the nature of that unique microbiota composition in ASD still eludes science. As these researchers concluded, "The complexity of the symptoms and the etiology of ASD coupled with the complexity of the microbiota and its functions has presented challenges in establishing the nature of an association between gut microbiota and ASD, pinning down whether a link even exists and for which individuals with ASD, and in producing a mechanistic understanding of the nature of this association."

Some of the reported changes in gut flora in autistics are thought to be related to frequent use in autistics of antibiotics, such as in treatment of otitis media. However, studies [84,85] have so far found no significant difference in gut microbiome between autistics and their neurotypical non-autistic siblings. As one study concluded, "Results did not indicate clinically meaningful differences between groups."

Nevertheless, glyphosate does appear to have the capability to alter the gut microbiome, Shehata et al. [79] tested bacteria in culture medium against various strengths of herbicide-formulated glyphosate. They found that most of the pathogenic bacteria they tested were resistant to glyphosate, while most of the beneficial bacteria were found to be moderately to highly susceptible. Thus, glyphosate appears to have a tendency to reduce beneficial species, perhaps contributing to the hyper-Westernized gut microflora described by researchers studying the subject of gut microflora in autism.

There are studies which hint at differences in gut microbiota between autistics and non-autistic non-siblings. Finegold et al. [78] in 2002 reported on a study of gastric, small bowel and stool samples from late onset autism patients compared to non-sibling controls. Special care was taken to capture and culture anaerobes. Many of these autism patients were on a gluten-free, casein-free diet. All had gastrointestinal symptoms, primarily diarrhea and/or constipation. All patients had received no antibacterial agents for at least 1 month prior to the study. Results indicated a definite difference between groups, i.e. "Children with autism had 9 species of Clostridium not found in controls, whereas controls yielded only 3 species not found in children with autism. In all, there were 25 different clostridial species found. In gastric and duodenal specimens; the most striking finding was total absence of non-spore-forming anaerobes and microaerophilic bacteria from control children and significant numbers of such bacteria from children with autism."

Limitations of study design in earlier autism research

Autism appears to be associated with relatively minor and difficult to visualize histologic manifestations, such as patchy disruptions of mini-column organization in brain development [86,87], as discussed above. Studies focused on larger scale defects in neurodevelopment such as malformations may miss such key localized findings. A study of sub-lethal dosing of rats with glyphosate [88] did document vacuolar changes in brain tissue but claimed to find no other changes, without referencing neuronal clustering or orderliness of mini-column arrangement of neurons or the like. In order to answer the question of whether a putative glycine-mimetic candidate molecule, such as glyphosate, might play a role in autism causation, it will be useful to design studies which have the potential to shed light on the relevant issues.

Risperidone, NMDA receptors, AMPA receptors

The first drug approved by the FDA for treatment of autism, risperidone, is the most widely used. Risperidone can have severe side effects, but can also prove effective in reducing tantrums, aggression and self-injury. The improvement is generally seen in approximately half the patients and can be dramatic, taking effect in a matter of weeks. Symptoms often return, however, when the drug is discontinued. Side effects of weight gain, sleepiness and high prolactin effects limit the use of risperidone.

For the purpose of the theory herein advanced, the mechanism of action of risperidone is relevant. Current theories of action tend to focus on risperidone and blockage of D2 and 5-HT2A receptors; however, the study by Choi et al. [89] suggests an action of risperidone in regard to other neuron receptors. These researchers compared 3 weeks of dosing at 3 different levels of risperidone in juvenile rats as compared to the same dosing in adults rats. They found "Risperidone (at 1.0 and 3.0 mg/kg/day) significantly decreased NMDA binding in caudate-putamen of juvenile and adult animals." In contrast, the same two doses of risperidone "decreased NMDA receptors in nucleus accumbens of juveniles and not adults."

They also found that "risperidone (at 1.0 and 3.0 mg/kg/day) increased AMPA receptors in medial prefrontal cortex and caudate-putamen of juvenile animals, whereas risperidone (at 3.0 mg/kg) increased AMPA receptors in caudate-putamen and hippocampus of adults." These findings fit the theory herein advanced that haphazard calcium inflow into neurons is key to autism. For example, such haphazard calcium inflow should theoretically be reduced when NMDA receptor activity is decreased. Risperidone success, in the study, was associated with a reduction of NMDA receptors. Also, the increase of AMPA receptors documented in the Choi et al. study of risperidone mechanism of action fits the theory herein advanced, because AMPA receptors within the brain are generally of the GluR2(R) type. The GluR2(R) receptor is known to admit sodium and potassium, while not permitting calcium to inflow.

Discussion

The available evidence appears to link research findings in autism to defects in early (including in utero) neurodevelopment [15]. Scientific studies are still ongoing to determine which governing factors for human neurodevelopment act at which steps to influence neuron migration and/or gene expression. However, in view of the rising incidence of autism diagnosis, we hold that it is time to consider the theory herein advanced that human fetal neurodevelopment might be altered by exposure to intermittent doses of a relatively small number of molecules of a putative harmful glycine-mimetic.

The theory that such a toxin is originating from environmental and/or dietary sources is supported by the finding that the brains of at least some of the control subjects in the studies by Suzuki et al. [13] and Stoner et al. [15] had findings similar to the findings in the brains of autistics. Similarly, the plasma amino acid changes documented by Aldred et al. [73] in autistics were present also in their family members. Similarly, the fact that studies of gut microbiota of autistics compared to gut microbiota of their non-autistic siblings [84] have not shown significant differences could be explained by the presence in food for both groups of a harmful glycine mimetic acting as a biocide.

Importantly, the theory herein advanced holds that an environmental or dietary sourced exposure to a human fetus of such a putative harmful glycine mimetic would likely not follow a standard dose response curve. We believe evidence [30] supports the view that the effects on neurodevelopment in the fetus of such theorized harmful glycine mimetic exposure are dependent on factors such as timing of exposure, and on location of molecular interactions. This theory, if proven, might have far reaching implications, such as precluding, at least for pregnant women, the supposed utility of safe allowable limits in food or on crops of the putative harmful glycine mimetic.

In essence, neurodevelopment is unlike other natural processes, in that a small number of molecules disrupting neurodevelopment at just the wrong time could have devastating autism-causing and long lasting outsized effects [9]. This viewpoint is valid, we believe, because fetal neurodevelopment is not a steady state process [9]. Rather, fetal neurodevelopment, especially in its early stages, has delicate and naturally choreographed steps [9,30] which appear inordinately sensitive to small disruptive insults. Such small disruptive intermittent events coming from a harmful glycine mimetic during

neurodevelopment could explain previously puzzling autism features such as the patchy cortical foci of disorganization [15] or the autistic brain's functional connectivity alterations, or the failure of proper development of the GABA switch [61], or the wide spectrum of symptoms in ASD.

We recognize that this paper is speculative, but we hope it will inspire others to conduct research to test the validity of our proposed hypothesis. If our ideas are validated, it is imperative for governments to take regulatory action against the practice of widespread glyphosate usage on food crops.

Acknowledgement

This research is supported in part by Quanta Computers, Taiwan, under the auspices of the Qmulus program.

References

1. Newschaffer CJ, Croen LA, Daniels J, Giarelli E, Grether JK, et al. (2007) The epidemiology of autism spectrum disorders. Annu Rev Public Health 28: 235-258.
2. Gardener H, Spiegelman D, Buka SL (2009) Prenatal risk factors for autism: comprehensive meta-analysis. Br J Psychiatry 195: 7-14.
3. Yuen RK, Thiruvahindrapuram B, Merico D, Walker S, Tammimies K, et al. (2015) Whole-genome sequencing of quartet families with autism spectrum disorder. Nat Med 21: 185-191.
4. Koller VJ, Furhacker M, Nersesyan A, MiÅ¡Ãk M, Eisenbauer M, et al. (2012) Cytotoxic and DNA-damaging properties of glyphosate and Roundup in human-derived buccal epithelial cells. Arch Toxicol 86: 805-813.
5. Freitag CM (2007) The genetics of autistic disorders and its clinical relevance: a review of the literature. Mol Psychiatry 12: 2-22.
6. Rapin I, Tuchman RF (2008) Autism: definition, neurobiology, screening, diagnosis. Pediatr Clin North Am 55: 1129-1146, viii.
7. Lyall K, Schmidt RJ, Hertz-Picciotto I (2014) Maternal lifestyle and environmental risk factors for autism spectrum disorders. Int J Epidemiol 43: 443-464.
8. Rutter M (2005) Incidence of autism spectrum disorders: changes over time and their meaning. Acta Paediatr 94: 2-15.
9. Arndt TL, Stodgell CJ, Rodier PM (2005) The teratology of autism. Int J Dev Neurosci 23: 189-199.
10. Rzhetsky A, Bagley SC, Wang K, Lyttle CS, Cook EH Jr, et al. (2014) Environmental and state-level regulatory factors affect the incidence of autism and intellectual disability. PLoS Comput Biol 10: e1003518.
11. Haar S, Berman S, Behrmann M, Dinstein I (2014) Anatomical Abnormalities in Autism? Cereb Cortex.
12. Shen MD, Nordahl CW, Young GS, Woolton-Gorges SL, Lee A, et al. (2013) Early brain enlargement and elevated extra-axial fluid in infants who develop autism spectrum disorder. Brain 136: 2825-2835.
13. Suzuki K, Sugihara G, Ouchi Y, Nakamura K, Futatsubashi M, et al. (2013) Microglial activation in young adults with autism spectrum disorder. JAMA Psychiatry 70: 49-58.
14. Niinomi Y, Ikeda M, Yamashita M, Ishida Y, Asai M, et al. (2013) Glyphosate-resistant Italian ryegrass (Lolium multiflorum) on rice paddy levees in Japan Weed Biology and Management 13: 31-38.
15. Stoner R, Chow ML, Boyle MP, Sunkin SM, Mouton PR, et al. (2014) Patches of disorganization in the neocortex of children with autism. N Engl J Med 370: 1209-1219.
16. Lopez-Hurtado E, Prieto J J (2008) A Microscopic Study of Language-Related Cortex in Autism Am J Biochem Biotech 4(2): 130-45.
17. Kirsch J, Betz H (1998) Glycine-receptor activation is required for receptor clustering in spinal neurons. Nature 392: 717-720.
18. Tang G, Gudsnuk K, Kuo SH, Cotrina ML, Rosoklija G, et al. (2014) Loss of mTOR-dependent macroautophagy causes autistic-like synaptic pruning deficits. Neuron 83: 1131-1143.
19. Smalley SL (1998) Autism and tuberous sclerosis. J Autism Dev Disord 28: 407-414.
20. Bolton PF, Park RJ, Higgins JN, Griffiths PD, Pickles A (2002) Neuro-epileptic determinants of autism spectrum disorders in tuberous sclerosis complex. Brain 125: 1247-1255.
21. Fombonne E (2003) Epidemiological surveys of autism and other pervasive developmental disorders: an update. J Autism Dev Disord 33: 365-382.
22. Johnson SC, Rabinovitch PS, Kaeberlein M (2013) mTOR is a key modulator of ageing and age-related disease. Nature 493: 338-345.
23. Wienecke R, Fackler I, Linsenmaier U, Mayer K, Licht T, et al. (2006) Antitumoral activity of rapamycin in renal angiomyolipoma associated with tuberous sclerosis complex. Am J Kidney Dis 48: e27-29.
24. Franz DN, Leonard J, Tudor C, Chuck G, Care M, et al. (2006) Rapamycin causes regression of astrocytomas in tuberous sclerosis complex. Ann Neurol 59: 490-498.
25. Neves-Pereira M, Müller B, Massie D, Williams JH, O'Brien PC, et al. (2009) Deregulation of EIF4E: a novel mechanism for autism. J Med Genet 46: 759-765.
26. Ehninger D, Silva AJ (2011) Rapamycin for treating Tuberous sclerosis and Autism spectrum disorders. Trends Mol Med 17: 78-87.
27. Butler MG, Dasouki MJ, Zhou XP, Talebizadeh Z, Brown M, et al. (2005) Subset of individuals with autism spectrum disorders and extreme macrocephaly associated with germline PTEN tumour suppressor gene mutations. J Med Genet 42: 318-321.
28. Gulati P, Gaspers LD, Dann SG, Joaquin M, Nobukuni T, et al. (2008) Amino acids activate mTOR complex 1 via Ca2+/CaM signaling to hVps34. Cell Metab 7: 456-465.
29. Palmieri L, Papaleo V, Porcelli V, Scarcia P, Gaita L, et al. (2010) Altered calcium homeostasis in autism-spectrum disorders: evidence from biochemical and genetic studies of the mitochondrial aspartate/glutamate carrier AGC1. Mol Psychiatry 15: 38-52.
30. Avila A, Nguyen L, Rigo JM (2013) Glycine receptors and brain development. Front Cell Neurosci 7: 184.
31. Kruger M, Schrödl W, Pedersen Ib, Shehata AA (2014) Detection of Glyphosate in Malformed Piglets. J Environ Anal Toxicol 4: 230.
32. Swanson NL, Leu A, Abrahamson J, Wallet B (2014) Genetically engineered crops, glyphosate and the deterioration of health in the United States of America. Journal of Organic Systems 9.
33. Laube B, Kuryatov A, Kuhse J, Betz H (1993) Glycine-glutamate interactions at the NMDA receptor: role of cysteine residues. FEBS Lett 335: 331-334.
34. Choudhury PR, Lahiri S, Rajamma U (2012) Glutamate mediated signaling in the pathophysiology of autism spectrum disorders. Pharmacol Biochem Behav 100: 841-849.
35. Cuscó I, Medrano A, Gener B, Vilardell M, Gallastegui F, et al. (2009) Autism-specific copy number variants further implicate the phosphatidylinositol signaling pathway and the glutamatergic synapse in the etiology of the disorder. Hum Mol Genet 18: 1795-1804.
36. Krey JF, Dolmetsch RE (2007) Molecular mechanisms of autism: a possible role for Ca2+ signaling. Curr Opin Neurobiol 17: 112-119.
37. Page LA, Daly E, Schmitz N, Simmons A, Toal F, et al. (2006) In vivo 1H-magnetic resonance spectroscopy study of amygdala-hippocampal and parietal regions in autism. Am J Psychiatry 163: 2189-2192.
38. Stobart JL, Anderson CM (2013) Multifunctional role of astrocytes as gatekeepers of neuronal energy supply. Front Cell Neurosci 7: 38.
39. Samsel A, Seneff S (2015) Glyphosate, pathways to modern diseases III: Manganese, neurological diseases, and associated pathologies. Surg Neurol Int 6: 45.
40. Cakmak, I, Yazici A, Tutus Y, Ozturk L (2009) Glyphosate reduced seed and leaf concentrations of calcium, manganese, magnesium, and iron in non-glyphosate resistant soybean. Eur Journal of Agronomy 31: 114-119.

41. Krüger M, Schrodl W, Neuhaus J, Shehata AA (2013) Field investigations of glyphosate in urine of Danish dairy cows J Environ Anal Toxicol 3: 1-7.
42. Abdullah MM, Ly AR, Goldberg WA, Clarke-Stewart KA, Dudgeon JV, et al. (2012) Heavy metal in children's tooth enamel: related to autism and disruptive behaviors? J Autism Dev Disord 42: 929-936.
43. Carl GF, Keen CL, Gallagher BB, Clegg MS, Littleton WH, et al. (1986) Association of low blood manganese concentrations with epilepsy. Neurology 36: 1584-1587.
44. Sohler A, Pfeiffer CC (1979) A direct method for the determination of manganese in whole blood. Patients with seizure activity have low blood levels. J Orthomol Psych 8: 275-280.
45. Volkmar FR, Nelson DS (1990) Seizure disorders in autism. J Am Acad Child Adolesc Psychiatry 29: 127-129.
46. Hyrc K, Handran SD, Rothman SM, Goldberg MP (1997) Ionized intracellular calcium concentration predicts excitotoxic neuronal death: observations with low-affinity fluorescent calcium indicators. J Neurosci 17: 6669-6677.
47. Cattani D, de Liz Oliveira Cavalli VL, Heinz Rieg CE, Domingues JT, Dal-Cim T, et al. (2014) Mechanisms underlying the neurotoxicity induced by glyphosate-based herbicide in immature rat hippocampus: involvement of glutamate excitotoxicity. Toxicology 320: 34-45.
48. Piton A, Gauthier J, Hamdan FF, Lafrenière RG, Yang Y, et al. (2011) Systematic resequencing of X-chromosome synaptic genes in autism spectrum disorder and schizophrenia. Mol Psychiatry 16: 867-880.
49. Dupont E, Hanganu IL, Kilb W, Hirsch S, Luhmann HJ (2005) Rapid developmental switch in the mechanisms driving early cortical columnar networks. Nature 439: 79-83.
50. Cossart R (2011) The maturation of cortical interneuron diversity: how multiple developmental journeys shape the emergence of proper network function? Curr Poin Neurobiol 21: 160-168.
51. Sauer JF, Bartos M (2011) Postnatal differentiation of cortical interneuron signalling. Eur J Neurosci 34: 1687-1696.
52. Wu H, Jin Y, Wei J, Jin H, Sha D, et al. (2005) Mode of action of taurine as a neuroprotector. Brain Res 1038: 123-131.
53. Wu JY, Wu H, Jin Y, Wei J, Sha D, et al. (2009) Mechanism of neuroprotective function of taurine. Adv Exp Med Biol 643: 169-179.
54. Leon R, Wu H, Jin Y, Wei J, Budhata C, et al. (2009) Protective function of taurine in glutamate-induced apoptosis in cultured neurons. J Neurosci Res 87: 1185-1194.
55. Hansen MG, Landmesser LT (2003) Characterization of the circuits that generate spontaneous episodes of activity in the early embryonic spinal cord. J Neurosci 23: 587-600.
56. Hansen MG, Landmesser LT (2004) Normal Patterns of Spontaneous Activity Are Required for Correct Motor Axon Guidance and the Expression of Specific Guidance Molecules Neuron Sept 43: 687-701.
57. Schmitz Y, Castagna C, Mrejeru A, Lizardi-Ortiz JE, Klein Z, et al. (2013) Glycine transporter-1 inhibition promotes striatal axon sprouting via NMDA receptors in dopamine neurons. J Neurosci 33: 16778-16789.
58. Hanson MG, Landmesser LT (2006) Increasing the frequency of spontaneous rhythmic activity disrupts pool-specific axon fasciculation and pathfinding of embryonic spinal motoneurons. J Neurosci 26: 12769-12780.
59. Di Martino A, Kelly C, Grzadzinski R, Zuo XN, Mennes M, et al. (2011) Aberrant striatal functional connectivity in children with autism. Biol Psychiatry 69: 847-856.
60. Avila A, Vidal PM, Dear TN, Harvey RJ, Rigo JM, et al. (2013) Glycine receptor Î±2 subunit activation promotes cortical interneuron migration. Cell Rep 4: 738-750.
61. Ben-Ari Y (2015) Is birth a critical period in the pathogenesis of autism spectrum disorders? Nat Rev Neurosci 16: 498-505.
62. Ghanizadeh A (2010) Possible role of caffeine in autism spectrum disorders, a new testable hypothesis. J Food Sci 75: ix.
63. Rungta RL, Choi HB, Tyson JR, Malik A, Dissing-Olesen L, et al. (2015) The cellular mechanisms of neuronal swelling underlying cytotoxic edema. Cell 161: 610-621.
64. Zhang HX, Lyons-Warren A, Thio LL (2009) The glycine transport inhibitor sarcosine is an inhibitory glycine receptor agonist. Neuropharmacology 57: 551-555.
65. Koh L, Zakharov A, Johnson M (2005) Integration of the subarachnoid space and lymphatics: Is it time to embrace a new concept of cerebrospinal fluid absorption?Cerebrospinal Fluid Res. 2: 6.
66. Louveau A, Smirnov I, Keyes TJ, Eccles JD, Rouhani SJ, et al. (2015) Structural and functional features of central nervous system lymphatic vessels. Nature 523: 337-341.
67. Preston JE, Segal MB, Walley GJ, Zlokovic BV (1989) Neutral amino acid uptake by the isolated perfused sheep choroid plexus. J Physiol 408: 31-43.
68. Schlessinger A, Wittwer MB, Dahlin A, Khuri N, Bonomi M, et al. (2012) High selectivity of the Î³-aminobutyric acid transporter 2 (GAT-, SLC6A13) revealed by structure-based approach. J Biol Chem 287: 37745-37756.
69. Napolioni V, Persico AM, Porcelli V, Palmieri L (2011) The mitochondrial aspartate/glutamate carrier AGC1 and calcium homeostasis: physiological links and abnormalities in autism. Mol Neurobiol 44: 83-92.
70. Rainey L, van der Walt JH (1998) The anaesthetic management of autistic children. Anaesth Intensive Care 26: 682-686.
71. Lemonnier E, Degrez C, Phelep M, Tyzio R, Josse F, et al. (2012) A randomised controlled trial of bumetanide in the treatment of autism in children. Transl Psychiatry 2: e202.
72. Hassan TH, Abdelrahman HM, El-Masry NR, Hashim HM, El-Gerby KM et al. (2013) Blood and brain glutamate levels in children with autistic disorder. Res Autism Spectrum Disorders 7: 541-548.
73. Aldred S, Moore KM, Fitzgerald M, Waring RH (2003) Plasma amino acid levels in children with autism and their families. J Autism Dev Disord 33: 93-97.
74. Randolph-Gips M, Srinivasan P (2012) Modeling autism: a systems biology approach. J Clin Bioinforma 2: 17.
75. Rudnan D, Smith RB 3rd, Salam AA, Warren WD, Galambos JT, et al. (1973) Ammonia content of Food. Am J Clin Nutrition 26: 487-490.
76. Peh WY, Chew SF, Ching BY, Loong AM, Ip YK (2010) Roles of intestinal glutamate dehydrogenase and glutamine synthetase in environmental ammonia detoxification in the euryhaline four-eyed sleeper. Bostrychus sinensis Aquat Toxicol 98: 91-98.
77. Bolte ER (1998) Autism and Clostridium tetani. Med Hypotheses 51: 133-144.
78. Finegold SM, Molitoris D, Song Y, Liu C, Vaisanen ML, et al. (2002) Gastrointestinal microflora studies in late-onset autism. Clin Infect Dis 35: S6-6S16.
79. Shehata AA, Schrödl W, Aldin AA, Hafez HM, Krüger M (2013) The effect of glyphosate on potential pathogens and beneficial members of poultry microbiota in vitro. Curr Microbiol 66: 350-358.
80. de Magistris L, Familiari V, Pascotto A, Sapone A, Frolli A, et al. (2010) Alterations of the intestinal barrier in patients with autism spectrum disorders and in their first-degree relatives. J Pediatr Gastroenterol Nutr 51: 418-424.
81. Heberling CA, Dhurjati PS, Sasser M (2013) Hypothesis for a systems connectivity model of autism spectrum disorder pathogenesis: Links to gut bacteria, oxidative stress, and intestinal permeability. Med Hypoth Mar 80: 264-270.
82. Wang L, Christophersen CT, Sorich MJ, Gerber JP, Angley MT, et al. (2012) Elevated fecal short chain fatty acid and ammonia concentrations in children with autism spectrum disorder. Dig Dis Sci 57: 2096-2102.
83. Kosenka E, Kaminsky Y, Stavroskaya IG, Felipo V (2000) Alteration of mitochondrial calcium homeostasis by ammonia-induced activation of NMDA receptors in rat brain in vivo Brain Research 880: 139-146.
84. Gondalia SV, Palombo EA, Knowles SR, Cox SB, Meyer D, et al. (2012) Molecular characterization of gastrointestinal microbiota of children with autism (with and without gastrointestinal dysfunction) and their neurotypical siblings. Autism Res 5: 419-427.

85. Finegold SM, Dowd SE, Gontcharova V, Liu C, Henley KE, et al. (2010) Pyrosequencing study of fecal microflora of autistic and control children. Anaerobe 16: 444-453.

86. Casanova MF, Buxhoeveden DP, Switala AE, Roy E (2002) Minicolumnar pathology in autism. Neurology 58: 428-432.

87. Krajmalnik-Brown R, Lozupone C, Kang DW, Adams JB (2015) Gut bacteria in children with autism spectrum disorders: challenges and promise of studying how a complex community influences a complex disease. Microb Ecol Health Dis 26: 26914.

88. Tizhe EV, Ibrahim ND, Fatihu MY, Onyebuchi II, George BD, et al. (2014) Influence of zinc supplementation on histopathological changes in the stomach, liver, kidney, brain, pancreas and spleen during subchronic exposure of Wistar rats to glyphosate. Comp Clin Path 23: 1535-1543.

89. Choi YK, Gardner MP, Tarazi FI (2009) Effects of risperidone on glutamate receptor subtypes in developing rat brain. Eur Neuropsychopharmacol 19: 77-84.