EMFs and Chemicals as the Main Drivers of the Autism Epidemic: Mechanisms of Action

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Autism
There are a number of researchers who have argued for autism being caused by microwave frequency EMFs, in part because of the difficulty in explaining the huge increase in incidence based on any other causal factor, or even set of factors.
Cellular DNA Damage

Free Radicals

Germ line Mutations Affecting Synapses

Synapse formation disruption including:
- Dendritic outgrowth
- Synapse formation
- Synapse maturation
- Synapse elimination
- MeCP2 function

[Ca2+]i

VGCCs

Low intensity Microwave/Lower freq. EMFs

Various chemicals

NMDA-R

[Ca2+]i

NO
ONOO(-)
Free radicals
Oxid. stress
NO/ONOO(-) cycle

Brain-gut axis
So how are non-thermal EMF effects produced? I stumbled onto the answer that explains most of them in 2012 and have published 6 papers documenting this mechanism:


Each of these papers show that microwave and lower frequency EMFs act to activate what are called voltage-gated calcium channels, such that EMF effects on the cells can be blocked by calcium channel blockers, drugs that are specific for blocking the VGCCs.


The VGCCs occur in the plasma membrane of cells, which when activated, open a channel allowing Ca2+ ions to flow into the cell. Most of the biological effects are produced by excessive calcium in the cell \([\text{Ca}^{2+}]_i\).
If autism is caused by EMF exposure to the brain, then one would predict that hyperactivity of the main L-type VGCC in the brain, $\text{Ca}_v1.2$ will cause autism. This has been shown to be true. A mutation in the main gene encoding this channel (CACNA1C) causes **Timothy syndrome**, which is characterized by autism and autism spectrum disorder (ASD) symptoms, as well as cardiac changes that are lethal a very low age. This mutation produces hyperactivity of the channel, because the mechanism that closes the channel in response to prolonged depolarization and calcium elevation, is dysfunctional, such that the channel stays open much longer, allowing much larger fluxes to flow into the cell. A derivative of this Timothy syndrome mutation also causes autism in transgenic mice with symptoms described as showing “markedly restricted, repetitive, and perseverative behavior, altered social behavior, altered ultrasonic vocalization, and enhanced tone-cued and contextual memory following fear conditioning.”
The Timothy syndrome mutation is not the only rare mutation that causes autism by causing excessive activity of the VGCCs.

<table>
<thead>
<tr>
<th>Gene mutation relating to autism</th>
<th>Effect</th>
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<tbody>
<tr>
<td>CACNA1C (Timothy syndrome), slow closing mutation</td>
<td>Causes autism, severe cardiac effects</td>
</tr>
<tr>
<td>Mutation in beta subunit of L-type VGCCs. causes slow closing of channel</td>
<td>Cause autism</td>
</tr>
<tr>
<td>Slow closing mutations in CACNA1F (very low expression in the brain)</td>
<td>Cause symptoms of autism</td>
</tr>
<tr>
<td>Mutations that produce a deficiency in the calcium-activated potassium channel (KCNMA1) – a channel that acts to help close the VGCCs</td>
<td>Cause autism</td>
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Clearly shows that excessive VGCC activity can cause autism!
Question: Is elevated VGCC activity involved in causing autism in the general population of autism individuals?

Answer is **yes**, based on genetic polymorphism studies. Genetic polymorphism studies done with the same gene that mutates to produce Timothy syndrome (CACNA1C) show that an allele of that gene which produces increased activity of the gene is associated with increased autism susceptibility – also has other widespread neuropsychiatric effects. In addition, genetic polymorphism studies of two T-type VGCCs alleles that produce increased activity also cause increased susceptibility to autism.

Argues strongly for broad role of the VGCCs in causing autism in the autism population – provides strong support, therefore, for microwave EMFs causing autism in the general population.
EMFs act via activation of voltage-gated calcium channels (VGCCs). We know this because in 26 different studies, using 5 different channel blockers, it has been shown that calcium channel blocker block or greatly lower EMF effects. Over 200 studies have been published showing increased calcium signaling and changes in calcium fluxes following EMF exposures.

In contrast, many pathophysiological effects of NO are mediated through its role as a precursor of peroxynitrite (ONOO-), leading to free radical generation and oxidative stress and also through excessive calcium signaling.
There have been many researchers that have argued that the failure to develop proper synaptic connections is the central defect that occurs in autism/ASDs:


McFadden & Minshew 2013 Evidence for *dysregulation of axonal growth and guidance in ASD*. Front Hum Neurosci Oct 22;7:671


Gai et al, 2012 Rare structural variation of *synapse and neurotransmission genes to autism*. Mol Psychiatry 17:402.


There have been many researchers that have argued that the failure to develop proper synaptic connections is the central defect that occurs in autism/ASDs:

The most convincing type of evidence for this is that mutations in genes encoding proteins with very specific roles in forming synapses, SHANK3 and NEUROLIGIN 3 & 4, cause autism. These studies clearly show that synaptic disruption is central to the production of the whole spectrum of symptoms causing autism.

Other important studies show that
1. Brains of autism patients (post-mortem) studies show many histological changes indicating synapse disruption.
2. Animal models of autism also show similar histological changes discussed in 1.
3. Brain dysfunction in autism patients including lowered connectivity also indicate synapse disruption.
Rodent studies showed many years ago, that the nervous system is THE most sensitive tissue in the body to low level microwave/lower frequency EMFs (Tolgskaya & Gordon, see below).

Synaptic connections in regions of the brain are disrupted (p.65-74, 97,113,121,136), with many of these studies showing deformation of spines near the ends of dendrites, spines known to have essential roles in forming synaptic connections.

With still more sessions of low-intensity irradiation, spines disappeared entirely (p. 70). At the extreme, some neurons are completely asynaptic (p.73). It can be seen from this, that non-thermal exposures well within current safety standards, can cause severe disruption of synapse formation in animals.

A very recent study showed a close linkage between synapse development and autism in the mouse (Schuster et al, NOMA-GAP/ARHGAP33 regulates synapse development and autistic-like behavior in the mouse. Mol Psychiatry 2015 Apr 14).

This study showed that a mutation that produces autism-like social behavior in the mouse, showed aberrant synapse development and aberrant dendritic spine morphology.
A recent study has shown that synaptic pruning deficits can produce autism-like symptoms in the mouse.

Tang et al (2014 Neuron) showed that mutations in the mTOR gene causes changes in synaptic pruning and also causes autism-like social behavioral deficits in the mouse.

This study demonstrates the complexities of the connections between synapse formation and autism – failure to form proper synapses and in addition, failure to remove inappropriate synapses (pruning) can each have roles in the development of autism symptoms.
EMFs act via activation of voltage-gated calcium channels (VGCCs). We know this because in 26 different studies, using 5 different channel blockers, it has been shown that calcium channel blocker block or greatly lower EMF effects. Over 200 studies have been published showing increased calcium signaling and changes in calcium fluxes following EMF exposures.

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So how can excessive VGCC activity acting through excessive [Ca2+]i perturb synapses during brain development? Excessive [Ca2+]i can impact four processes involved in synaptic connections in the developing nervous system.

- The growth of processes from neurons towards the proper sites.
- Subsequent formations of the first synapses.
- Further formation of synapses as well as breakages of some synaptic connections.
- MeCP2 function (discussed in the following slide)

Each of these processes requires appropriate calcium control and is disrupted by excessive intracellular calcium!! (Reviewed in Cohen & Greenberg, Annu Rev Cell Dev Biol 2008;24:183-209; Lohmann C, 2009, Prog Brain Res 175:443)

Lohmann argues that dysfunction of each of these processes has an essential role causing autism, consistent with the earlier arguments supporting an essential role of disruption of synapse formation in causing autism.

Long term potentiation, a process for strengthening synaptic connections in the brain, is also [Ca2+]i dependent.
There is a 5th process that impacts synapse formation in the developing brain, one that is partly overlapping the processes discussed in the previous slide and one that is partly independent of them. It involves the MeCP2 gene and the MeCP2 protein (which is a calcium regulated transcriptional regulator) produced by the gene.

Mutations that knock out function of MeCP2 gene are known to cause Rett syndrome, considered part of the autism spectrum. This produces changes in regulation of genes that have roles in synapse development and therefore disrupts synapse development.

Calcium-dependent phosphorylation of the MeCP2 protein, lowers its activity and causes the protein to behave as if it were a mutant protein.

So this is another way in which elevated [Ca2+]i can disrupt normal synapse development!
We started this discussion with the parallel increase between microwave EMF exposures and the incidence and autism. Are there other epidemiological &/or clinical observations that also suggest a linkage?

In Brick Township, New Jersey where a radar station leads to substantial levels of exposure over the town, the autism incidence was somewhat unusually high. Bertrand J, et al. Pediatrics 2001 108(5):1155.

Dr. Dietrich Klinghardt reported that the EMF intensity where pregnant mothers slept who produced autism children was over 20 times higher than for mothers who produced normal children – with totally non-overlapping values. Small study but with high statistical significance!

Dr. Tori Jelter (and I think Dr. Klinghardt) each reported that their autism patients showed very substantial improvements in symptoms, when moved into an environment with much lower field exposures.

No doubt we need many more similar studies.
Cellular DNA Damage

Germ line Mutations Affecting Synapses

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Free Radicals

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Brain-gut axis
4 types of evidence that microwave EMFs act via activation of VGCCs

Rare mutations in 4 genes that produce excessive VGCC activity each cause autism (4)

Genetic polymorphism in CACNA1C gene producing elevated activity produces increased susceptibility to autism and genetic polymorphisms in 2 T-type genes also act similarly (3)

Central cause of autism thought to be disruption of normal synaptic development process (3)

Supported by neurophys changes in autism patients
Supported by changes in brain structures (post-mortem) in autism
Supported by changes in brain structures in rodent autism models

Rodent brains very sensitive to microwave EMFs, with changes including many changes in synaptic connections (2)
Mutations in Shank3 and two neuroligin genes (specific roles in forming synapses) each cause autism in humans (3)

Autism mouse models produced by mutations that cause synaptic disruption (1)

Excessive [Ca2+]i causes dysfunction of 4 different mechanisms each of which have important roles in forming proper synapses (4)

Dysfunction of each of the 4 (immediately above) have roles in autism (4)

4 types of epidemiological or clinical observation studies (4).

Total of 32 types of evidence supporting the sequence shown at the top of slide, 16 on previous slide and 16 shown on this slide.
Cellular DNA Damage → Free Radicals → VGCCs → [Ca2+]i → NMDA-R → Various chemicals

Low intensity Microwave/ Lower freq. EMFs → [Ca2+]i → NO ONOO(-) Free radicals Oxid. stress NO/ONOO(-) cycle

Germ line Mutations Affecting Synapses → Synapse formation disruption including:
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Brain-gut axis
The most impressive, scientific studies of autism are undoubtedly the genetic studies. Such studies found many *de novo* mutations occurring in autism patients – mutations not occurring in their parents – with about 12 – 15% of such patients carrying such mutations.

When similar mutations are produced in transgenic mice, they produce autism-like symptoms, strongly suggesting that they help cause autism in humans.

Autism patients carrying such mutations usually show what are called syndromic forms of autism, where their symptoms vary from those of others, depending on the mutation. Most of these mutations impact synapse formation and function, consistent with a central synaptic role in autism.

In terms of DNA changes, three different types of mutations are common: Copy number mutations, larger chromosomal rearrangements and point mutations that disrupt gene function.
These findings produce a challenge in explaining the autism epidemic. When similar mutations are produced in transgenic mice, they produce autism-like symptoms, strongly suggesting that they help cause autism in humans. Because such mutations occur in a minority, albeit substantial minority of autism patients, it is very unlikely that they can be the only cause of the epidemic. On the other hand with a 150-fold or so increase in autism, it seems clear that they are common enough that they must contribute to the epidemic. Most of these mutations impact synapse formation and function, consistent with a central synaptic role in autism. In terms of DNA changes, three different types of mutations are common: copy number mutations, larger chromosomal rearrangements and point mutations that disrupt gene function.
It is known that microwave frequency EMFs cause changes to the DNA of cells, including single strand and double strand DNA breaks and 8-OHdG. It is also known that EMFs have impacts on the germ lines, including DNA changes in sperm and there are animal studies reporting increased mutation rates following EMF exposure.

The types of DNA damage described in the first paragraph, above can cause the types of mutations found in autism patients. Accordingly, I propose that many of these de novo mutations are caused by EMF exposures in the parents. For this reason, exposures to the parents may contribute the autism epidemic. Therefore EMF exposures to the parents may have some causal role in the autism epidemic, not just exposures during the perinatal period or the period following it.
Cellular DNA Damage → Free Radicals → VGCCs → [Ca2+]i → Various chemicals → NMDA-R → Brain-gut axis

Low intensity Microwave/Lower freq. EMFs → VGCCs → [Ca2+]i → NO/ONOO(-) Free radicals Oxid. stress NO/ONOO(-) cycle

Germ line Mutations Affecting Synapses → Synapse formation disruption including:
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There are a whole series of similarities between the NMDA receptors and the L-type VGCCs:

1. Both open up an ion channel when activated.
2. Both channels stay open are relatively long time period compared with other channels.
3. Both allow substantial amounts of calcium to flow into the cell.
4. The effects of both are thought to be mediated by excessive cytoplasmic calcium.
5. Both lead to the production of large amounts of nitric oxide, due to the action of two calcium-dependent nitric oxide synthases, with the nitric oxide often leading in both to production of peroxynitrite.
6. Both have been shown to be able to stimulate long-term potentiation, the process in the central nervous system involved in learning and memory by producing neural sensitization.
Genetic polymorphism studies on NMDA receptors and autism:

There are genetic polymorphisms in both the GRIN2A gene, which produce elevated NMDA activity, apparently cause increased susceptibility to autism.

Similarly there is a common allele of the GRIK2 gene, that produces increased activity of the kainate (glutamate) receptor that works along with the NMDA receptor, and also apparently produces increased susceptibility to autism.
The problem with explaining the autism epidemic via these chemicals is that three classes of these chemicals:

- Organic Solvents and other Sensory Irritants
- The organophosphorus and carbamate pesticides
- The organochlorine pesticides

Each of these had their largest increases in synthesis in the 30 years following World War II, before there were substantial increases in the incidence of autism. So these alone are unlikely to have major roles here, although they may act synergistically along with EMF exposures.

What about other chemicals where we may have had substantial increases since that time?
Chemical Action in MCS

- Organophosphorus/carbamate pesticides
- Organochlorine pesticides
- Acetylcholinesterase
- Acetylcholine
- Muscarinic activity
- GABAA receptors
- Nitric oxide
- TRPV1, TRPA1, other TRP receptors
- NMDA receptor activity
- H₂S
- Pyrethroid pesticides
- Glutamate transport
- Sodium channels
- Hg
- MeHg
- Glutamate
- [Ca²⁺]i
<table>
<thead>
<tr>
<th>Chemical</th>
<th>Action</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen acting via NAPQI</td>
<td>Reacts with thiol groups, may activate TRP receptors, inactivate glutamate transport, impact mitochondria leading to mitochondrial calcium release</td>
<td>Proposed by Dr. William Shaw to be important in autism.</td>
</tr>
<tr>
<td>Type 2 pyrethroids</td>
<td>May lead to increases in both NMDA and VGCC activity</td>
<td>Started production in late 1970s; Not previously proposed for autism role, to my knowledge</td>
</tr>
<tr>
<td>Glyphosate</td>
<td>Increases both NMDA and VGCC activities, according to Brazilian group. Acts in part via elevated glutamate.</td>
<td>Autism role proposed by Dr. Stephanie Seneff due to completely different proposed mechanisms; parallel increase between glyphosate and autism</td>
</tr>
<tr>
<td>Mercury; mercurials</td>
<td>Act via methyl/ethyl mercury through thiol reactions to lower glutamate transport and raise TRPC4/5 activities</td>
<td></td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td>Produce increased NMDA activity</td>
<td>New for autism role</td>
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</table>
Both the NMDA receptors and the VGCCs act to partially depolarize the plasma membrane and they also are both activated, to some extent, by such depolarization. They both produce rises in \([\text{Ca}^{2+}]_i\). Consequently, stressors that activate one of these may act synergistically with stressors that raise the other, to raise \([\text{Ca}^{2+}]_i\) and in this way cause autism.

It is likely, therefore, that both microwave EMFs and chemical exposures have substantial roles in causing the autism epidemic. It is my view that the EMFs are probably more important than are the chemicals, but I could be wrong about this; furthermore possible synergism means that simple additive roles cannot be assumed.

Prevention should focus on avoiding both types of exposures.
Cellular DNA Damage → Germ line Mutations Affecting Synapses → Synapse formation disruption including:
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Free Radicals → [Ca2+]i → VGCCs → Low intensity Microwave/Lower freq. EMFs → NMDA-R → Various chemicals

NO ONOO(-) Free radicals Oxid stress NO/ONOO(-) cycle → Brain-gut axis
The last issue to be discussed here is the NO/ONOO(-) cycle, which was proposed, in my book, to be elevated in autism. Pall ML 2007, Explaining “Unexplained Illnesses”: … .


The cycle is a primarily local biochemical vicious cycle with a series of elements elevated through their mutual interactions.
Each of the elements of the cycle have been shown to have roles in causation of autism except possibly the TRP receptors where I am unaware of any data.

And lowering NO/ONOO(-) cycle elements is reported to be useful in therapy. For example, James et al reported that reduced folates and high concentrations of vitamin B12 are useful in autism treatment (Am J Clin Nutr 2009;89:425). This may be because reduced folate raises the powerful peroxynitrite scavenger 5-methyltetrahydrofolate (5-MTHF) (see Rezk, FEBS Lett 2003;555:601–605; Antoniades, Circulation 2006;114:1193–1201). It may also be because the hydroxocobalamin form of B12 is a potent scavenger of NO and superoxide. (Has previously interpreted in terms of methylation).

Tetrahydrobiopterin (BH4) supplements have been shown to be useful in autism treatment (see Frye, N Amer J Med Sci 2014;7:93-96).
Raising Nrf2 activity may be a general way of lowering the NO/ONOO(-) cycle.


In general, then autism should be treated by lowering the NO/ONOO(-) cycle as well as by avoiding both EMF and chemical causal exposures.

Prevention should focus mainly on avoiding both EMF and chemical causal exposures.
Healthful diets incl. Traditional Mediterranean & Okinawan

Probable Paleolithic Diet

Many Modern Diets

Many phenolic antioxidants
Isothiocyanates
Allium sulfur compounds
DHA, EPA (Omega-3s)
Carotenoids
Terpenoids
γ, δ-tocopherols, tocotrienols

Other health promoting factors:
- Low level oxid. stress (hormesis)
- Exercise
- Calorie restriction

Possible Increased Lifespan & Healthspan

Chronic diseases of oxid. stress, inflammation, mitochondrial dysfunction including NO/ONOO⁻ cycle diseases

Nrf2

Transcriptional stimulation ~500 genes

Antioxidant proteins
Mitochondrial biogenesis/energy metabolism
Detoxification, carbon-cont. toxicants, toxic metals
Autophagy
Lowered inflammation
How then can we prevent or treat autism:

Avoid exposures wherever possible to both EMFs and chemicals. The most critical exposures are likely to be during pregnancy and the months following birth. Still, EMF exposures to the father may be important for years before birth due to mutations that can be generated in the germ line cells.

Raise Nrf2 through diet, nutritional supplements and possible drugs.

Some other nutrients may be useful including 5-methyltetrahydrofolate (scavenges peroxynitrite), the hydroxocobalamin form of B12 (scavenges both NO and superoxide). Magnesium may be useful because of its lowering NMDA activity and also acts more modestly to lower VGCC activity. There may be behavioral approaches which may act in the child to normalize the synaptic structure and function.
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