Multiple Chemical Sensitivity (MCS): Chemical action, etiologic mechanism and treatment

Electromagnetic Field Hypersensitivity (EHS): EMF action and apparent etiologic mechanism

Martin L. Pall
Professor Emeritus of Biochemistry and Basic Medical Sciences
Washington State University
thetenthparadigm.org
martin_pall@wsu.edu
Much of the evidence that I will be discussing here comes from 4 of my publications:


Each of the classes of chemicals implicated in initiating cases of multiple chemical sensitivity can act to raise NMDA activity in the body.
Chemical Action in MCS

Organophosphorus/carbamate pesticides
- acetylcholinesterase
- acetylcholine
- muscarinic activity

Organochlorine pesticides
- GABAA receptors
- nitric oxide

Organic solvents
- TRPV1, TRPA1
- other TRP receptors

H₂S

Pyrethroid pesticides
- Sodium channels
- Glutamate transport

Hg

MeHg

Glutamate

NMDA receptor activity
The organic solvents and related compounds are thought to include most of a diverse set of compounds known as sensory irritants including alkanes, alkylbenzenes, halogenated benzenes, alcohols, ketones, ethers, aldehydes including formaldehyde, isocyanates, and even chlorine and other oxidants. It can be seen from this, that this group of compounds are extraordinarily diverse. Much of the sensory irritant mechanism has been shown to be mediated through the TRPA1 receptor.
Six other observations supporting an NMDA role in MCS:

1. MCS patients are sensitive to monosodium glutamate and glutamate is the physiological agonist of the NMDA receptors.
2. An allele of the CCK-B receptor gene that produces increased NMDA activity is associated with increased prevalence in two studies and therefore incidence of MCS.
3. The NMDA antagonist dextromethorphan is reported from clinical observations to produce lowered response to chemical exposures in MCS patients.
4. Bell and others have proposed that neural sensitization has a key role in MCS and the probable mechanism for such neural sensitization, called long-term potentiation, is known to involve increased NMDA activity.
5. Elevated NMDA activity has been shown to play an essential role in several animal models of MCS.
6. Elevated NMDA activity appears to play a role in such related illnesses as fibromyalgia, chronic fatigue syndrome and post-traumatic stress disorder, with the most extensive evidence for such a role being found in fibromyalgia (Pall, 2006 and 2007a).

Compelling evidence for a common toxicological response
<table>
<thead>
<tr>
<th>Gene</th>
<th>Study</th>
<th>Function of encoded enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>PON1</td>
<td>H; M</td>
<td>Detoxification of organophosphates</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>M</td>
<td>Hydroxylation</td>
</tr>
<tr>
<td>NAT2</td>
<td>M; S</td>
<td>Acetylation</td>
</tr>
<tr>
<td>GSTM1</td>
<td>S</td>
<td>Produce glutathione for conjugation</td>
</tr>
<tr>
<td>GSTT1</td>
<td>S</td>
<td>Glutathione conjugation</td>
</tr>
<tr>
<td>GSTP1</td>
<td>S</td>
<td>Glutathione conjugation</td>
</tr>
<tr>
<td>UGT1A1</td>
<td>M&amp;S</td>
<td>Glucuronidation of chemicals</td>
</tr>
</tbody>
</table>


Note: of the Schnakenberg (S) studies, one gene had p<10^{-3}, two had p<10^{-4} and the gene studied in the M&S study had p<10^{-4}. The p for all four of these taken together is p<10^{-15}.

Note 2: Replication in studies of different populations will depend on the relevant chemical exposures of the different populations!
There are other pathways along which toxicants can act to produce excessive NMDA activity, including those acting to produce lowered mitochondrial activity. Among the mitochondrial/energy metabolism toxicants that have been shown to act at least in part via excessive NMDA activity are: MPTP, rotenone, cyanide (although some of its effects increasing NMDA activity are through another pathway of action), carbon monoxide and hypoxia.
In summary, we have, then a vast array of TAVENAs (toxicants/toxins in the body that each act to trigger a common toxic end point- excessive NMDA activity). These appear to include:

- A vast array of organic solvents & related compounds including sensory irritants
- The three major classes of insecticides
- Several herbicides
- Several fungicides
- Several toxic metals
- Four classes of antibiotics
- A large array of liver toxicants/toxins
- Several mitochondrial toxicants/toxins
- Several tropical fish/shellfish toxins
- Several additional toxicants
## Parkinson’s initiators

<table>
<thead>
<tr>
<th>Agent</th>
<th>NMDA?</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>maneb</td>
<td>yes</td>
<td>Primarily via glutamate transport</td>
</tr>
<tr>
<td>paraquat</td>
<td>yes</td>
<td>Complex mechanism</td>
</tr>
<tr>
<td>Organic solvents</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>Organophos. pesticides</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>Organochlorine pesticides</td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td>MPTP</td>
<td>yes</td>
<td>Mitochondrial mechanism</td>
</tr>
<tr>
<td>rotenone</td>
<td>yes</td>
<td>Mitochondrial mechanism</td>
</tr>
<tr>
<td>manganese</td>
<td>yes</td>
<td>Requires high doses</td>
</tr>
<tr>
<td>BMAA</td>
<td>yes</td>
<td>Reacts with CO₂ to form compound that activates NMDA &amp; AMPA/kainate receptors; initiates ALS-Parkinson’s dementia complex</td>
</tr>
<tr>
<td>Pyrethroid pesticides</td>
<td>yes</td>
<td>Only recently documented</td>
</tr>
</tbody>
</table>

Most of these have been studied in animal models of PD and their causal role is clearly established using such models.
However there are many other chronic diseases where cases can be initiated by toxicants acting to produce excessive NMDA activity, including not only MCS and Parkinson’s disease, but also Alzheimer’s, amyotrophic lateral sclerosis (ALS), multiple sclerosis, tinnitus, asthma, myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS), autism and epilepsy.

Each of these has also been proposed by the author to be caused by what is called the NO/ONOO- cycle, a primarily local biochemical vicious cycle which, depending on where it is localized in the body, may be able to cause many different chronic inflammatory diseases. We outline, here, the properties of the NO/ONOO- cycle in the context of its proposed role in MCS.
NMDA receptor activation

channels allow calcium entry into cell

nNOS and eNOS activation

nitric oxide increase

react with superoxide to form peroxynitrite
We think that the etiologic mechanisms of MCS are centered on two interrelated mechanisms:

1. What is called the NO/ONOO- cycle, a primarily local biochemical vicious cycle that is initiated by various triggers, including those acting via increased NMDA activity, and propagates itself over time.

2. And another related mechanism proposed to be involved in MCS by Dr. Iris Bell and by others, neural sensitization caused by what is known as long-term potentiation. This can also involves NMDA receptor activity and several other mechanisms that are part of the NO/ONOO- cycle. Both 1 and 2 are discussed on some detail in my MCS toxicology review.

Let us first discuss the NO/ONOO- cycle.
Five Principles

1. Cases can be initiated by short-term stressors that increase cycle elements.
2. The chronic phase of illness is produced by the NO/ONOO- cycle. It is predicted, therefore, that the cycle elements will be elevated in the chronic phase of illness.
3. The symptoms and signs of illness must be generated by one or more elements of the cycle.
4. The basic mechanism of the cycle is local and will be localized to different tissues in different individuals. The reason for this primarily local nature is that the three compounds involved, NO, superoxide and ONOO-, have limited half lives in biological tissues. And the mechanisms of the cycle, those various arrows, act at the level of individual cells. This allows for great variations in tissue distribution from one patient to another, producing a huge spectrum of illness. The point here is not that there are no systemic changes, clearly there are, but rather that the primarily local mechanisms can generate great variation in diagnosis and in the symptoms and signs, from one individual to another.
5. NO/ONOO- cycle diseases should be treated by down-regulating the NO/ONOO- cycle biochemistry, rather than by symptomatic relief. In other words, we should treat the cause, rather than the symptoms.
There are 34 distinct, mechanisms that currently make up the NO/ONOO- cycle models as it was shown in the preceding figures. These are all copied on subsequent slides and are all documented in my pulmonary hypertension, NO/ONOO- cycle review. Of those 31 have reported substantial pathophysiological roles. I have added two additional mechanisms (35&36) which will be discussed here later.

Thus the only thing truly novel about the NO/ONOO- cycle, is that when these mechanisms are put into juxtaposition with each other, as they have been in the preceding figures, they serve collectively to integrate and explain a vast array of data about a large number of human diseases.
1. Extremely rapid, diffusion limited reaction between nitric oxide (NO-) with superoxide (OO-), forming peroxynitrite (ONOO-).
2. Peroxynitrite, a potent oxidant, can act mainly through its breakdown products to increase the activity of the transcription factor NF-kappaB.
3. Peroxynitrite breaks down both before and after reaction with carbon dioxide into the following free radicals, hydroxyl (HO·), carbonate (CO3·) and NO2 radical (NO2·), each of which are responsible for a number of consequences produced by peroxynitrite.
4. Peroxynitrite being a potent oxidant produces oxidative stress, an imbalance between oxidants and antioxidants.
5. Oxidative stress also produces increases in NF-kappaB activity.
6. NF-kappaB produces increased transcription of the inducible nitric oxide synthase (iNOS), a gene whose transcription is known to be stimulated by NF-kappaB elevation.
7. NF-kappaB also stimulates the transcription of several inflammatory cytokines, including IL-1β, IL-6, IL-8, TNF-α, and IFNγ.
8. Each of the five cytokines listed in 7 above, act directly and/or indirectly to stimulate the transcription of the iNOS gene, acting in some cases via the double headed arrow linking it to NF-kappaB.
9. When iNOS is induced, it produces large amounts of NO.
10. Peroxynitrite inactivates the calcium-ATPase, leading to increased levels of intracellular calcium.
11. Other oxidants also react with and inactivate the calcium-ATPase as well.
12. Large increases in intracellular calcium raise intramitochondrial calcium, which if large, lead to increased superoxide generation in the mitochondria and in some cases to apoptotic cell death.
13. Lowered energy metabolism (decreased energy charge/ATP) also lowers calcium-ATPase activity, leading to increased levels of intracellular calcium.
14. Intracellular calcium stimulates the nNOS and eNOS forms of nitric oxide synthase, both of which are calcium dependent enzymes.
15. Increased nNOS and eNOS activity both produce increased NO synthesis.
16. Peroxynitrite oxidizes tetrahydrobiopterin (BH4), depleting BH4 levels.
17. BH4 depletion produces partial uncoupling of the three NO synthases, such that some of these enzymes produce superoxide in place of NO. Because of the very rapid reaction of these two compounds to produce peroxynitrite, this partial uncoupling is expected to produce an increase in peroxynitrite production.
18. Nicking of nuclear DNA by hydroxyl and carbonate radicals, can produce a massive stimulation of poly ADP-ribosylation of chromosomal proteins, leading, in turn to a massive depletion of NAD/NADH pools, because NAD is the substrate for such poly ADP-ribosylation. NADH depletion lowers, in turn, ATP production in the mitochondrion.
19. Other changes causing ATP depletion come from a cascade of events occurring within the mitochondrion. The cascade starts with NO, possibly produced by mitochondrial NO synthase (mtNOS which is thought to be largely a form of nNOS), with NO binding to cytochrome oxidase, competitively inhibiting the ability of molecular oxygen to bind. This inhibits the ability of cytochrome oxidase to serve as the terminal oxidase of the mitochondrial electron transport chain.
20. The action of NO in 18 above, produces increase superoxide production by the electron transport chain.
21. Peroxynitrite, produced from the combination 18 and 19 above, also acts to produce increased superoxide from the electron transport chain.
22. Peroxynitrite, superoxide and their products lead to lipid peroxidation of the cardiolipin in the inner membrane of the mitochondrion. Cardiolipin is highly susceptible to such peroxidation, because most of the fatty acids that make up its structure in mammals are polyunsaturated fatty acids, which are much more susceptible to peroxidation than are other fatty acids.

23. Cardiolipin peroxidation leads to lowered activity of some of the enzymes in the electron transport chain, leading to further lowering of ATP synthesis.

24. Cardiolipin peroxidation also leads to increased superoxide generation from the electron transport chain in the mitochondrion.

25. Peroxynitrite produces inactivation of the mitochondrial superoxide dismutase (Mn-SOD), leading in turn to increased superoxide levels in the mitochondrion.

26. Peroxynitrite, superoxide and nitric oxide all inactivate or inhibit the aconitase enzyme, lowering citric acid cycle activity and subsequent ATP synthesis.

27. Oxidative stress leads to oxidation of cysteine residues in the enzyme xanthine reductase, converting it into xanthine oxidase which produces superoxide as a product, thus increasing superoxide generation.

28. Increased activity of the enzyme NADPH oxidase, which produces superoxide as a product, is an important part of the inflammatory cascade, and contributes, therefore, to the cascade by producing increased superoxide.

29. Activity of the NMDA receptors, allow calcium influx into the cell, raising intracellular calcium levels.

30. Activity of transfer receptor potential (TRP) receptors also allows calcium influx into the cell, again raising intracellular calcium levels, presumably leading to increased nitric oxide production.
31. The main physiological agonist of the NMDA receptors is glutamate whose extracellular concentration is lowered after release, by energy dependent transport. It follows that ATP depletion produces increased NMDA stimulation by lowering glutamate transport.

32. The activity of the NMDA receptors is also greatly increased by ATP depletion within the cells containing the NMDA receptors. The mechanism here is that the ATP depletion lowers the electrical potential across the plasma membrane, which produces, in turn, increased susceptibility of the NMDA receptors to stimulation.

33. Three of the TRP group of receptors have been shown to be stimulated by increased superoxide and/or oxidative stress or their downstream consequences, these being the TRPV1, TRPA1 and TRPM2 receptors, with the increased TRPV1 and TRPA1 activity being produced in part through the oxidation of cysteine residue side chains. Several TRP receptors are also activated by nitric oxide mediated nitrosylation.

34. TRPV1, TRPA1 and probably several other TRP group receptors, receptor stimulation has each been repeatedly shown to lead to increased NMDA activity, with neurons containing these TRP family of receptors acting in part by releasing glutamate, the major physiological NMDA agonist.

35. Activation of voltage-gated calcium channels (VGCCs) is produced by partial depolarization of the plasma membrane that is produced by mitochondrial dysfunction.

36. Such VGCC activation, leads, to increased intracellular Ca^{2+} levels.
The NO/ONOO- cycle provides explanations of how chemically caused excessive NMDA activity can produce MCS with its chronic local sensitivity to chemical exposure. The local elevation of the NO/ONOO- cycle in regions of the body susceptible to chemically-caused NMDA activation, will be expected to produce, at least part of the sensitivity response.
It should be noted, however, that chemicals are not the only stressors that can initiate cases of apparent NO/ONOO-cycle diseases. They may also be initiated by infections (probably acting via increased inflammation), by physical trauma especially to the central nervous system and by psychological stress (probably both acting via excessive NMDA activity) and by electromagnetic field exposure (probably acting via increased intracellular calcium). Some initiating chemicals may act independently of the NMDA receptors.
One of the big breakthroughs in our understanding of MCS came from a comparison of the NO/ONOO- cycle model of these illnesses with the neural sensitization model of MCS developed by Dr. Iris Bell (M.D., Ph.D., at the University of Arizona). Bell argued that the most important mechanism of MCS was neural sensitization in the hippocampus region of the brain. This is the same region that has key functions in learning and memory. The idea Bell developed was that the synapses in the brain, the contacts between neurons by which one stimulates another, may become both hypersensitive and hyperactive in response to chemical exposure. The basic idea here is that this process of neural sensitization which is involved on a very selective basis in learning and memory, appears to be activated massively in MCS.
The main mechanism of neural sensitization is known as long term potentiation (LTP). LTP is known to involve increased NMDA receptor activity, increased intracellular calcium nitric oxide and also superoxide. So one immediately sees major connections between the NO/ONOO- cycle mechanism and the neural sensitization mechanism developed by Bell. So by having chemicals producing increased NMDA activity, one can see how they could greatly stimulate the long term potentiation mechanism. Several of the elements of the NO/ONOO- cycle have roles in LTP, including NMDA activity, intracellular calcium, nitric oxide and superoxide.

LTP has been studied predominantly in the brain and spinal cord. It has been suggested to occur in some other tissues with NMDA receptors, but this has not been clearly demonstrated.
MCS has apparent sensitivity responses, not only coming from the brain, but also from upper and lower respiratory tract regions, from the skin, GI tract, eye and sometimes other tissues. Because we are unsure whether LTP occurs in these peripheral tissues, we are unsure whether it can contribute to sensitivity responses in those peripheral tissues. But, in general, it seems likely that MCS sensitivity involves the NO/ONOO\(^{-}\) cycle in these various tissues and also LTP in the central nervous system (and possibly elsewhere?).

Some other mechanisms may contribute to chemical sensitivity:
Nitric oxide (NO), acting to inhibit cytochrome P450 metabolism producing slowed detoxification and therefore possible increased sensitivity to some chemicals metabolized in this way.
Oxidants lead to increased TRPV1 and TRPA1 activity, leading to increased sensitivity to chemicals acting via these receptors.
Peroxynitrite, producing breakdown of the blood brain barrier, leading to increased chemical access to the brain.
Now, let’s switch over to the effects of electromagnetic fields (EMFs) on our biology and medicine!!

There has been a great puzzle about how EMFs can influence our biology, for better or for worse. These EMFs are composed of low energy photons, with energy per photon too low to influence the chemistry of the body! How can they influence our biology through non-thermal effects? And yet there is a substantial literature reporting that they do.

I have recently solved this important puzzle. EMFs act to influence the voltage across plasma membranes of cells, thus activating voltage-gate calcium channels. And it is the downstream effects of the increased intracellular Ca^{2+} that leads to the biological effects of EMF exposure. I will discuss first some of the evidence supporting this mechanism and will discuss later how this may lead to electromagnetic hypersensitivity.
<table>
<thead>
<tr>
<th>Ref #</th>
<th>EMF type</th>
<th>Calcium channel</th>
<th>Cell type or organism</th>
<th>Response measured</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Pulsed magnetic fields</td>
<td>L-type</td>
<td>Human lymphocytes</td>
<td>Cell proliferation; cytokine production</td>
</tr>
<tr>
<td>3</td>
<td>Static magnetic field (0.1 T)</td>
<td>L-type</td>
<td>Human polymorphonuclear leukocytes</td>
<td>Cell migration; degranulation</td>
</tr>
<tr>
<td>5</td>
<td>ELF</td>
<td>L-type</td>
<td>Rat chromaffin cells</td>
<td>Differentiation; catecholamine release</td>
</tr>
<tr>
<td>6</td>
<td>Electric field</td>
<td>L-type</td>
<td>Rat and mouse bone cells</td>
<td>Increased Ca2+, phospholipase A2, PGE2</td>
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<td>7</td>
<td>50 Hz</td>
<td>L-type</td>
<td>Mytilus (mussel) immunocytes</td>
<td>Reduced shape change, cytotoxicity</td>
</tr>
<tr>
<td>8</td>
<td>50 Hz</td>
<td>L-type</td>
<td>AtT20 D16V, mouse pituitary corticotrope-derived</td>
<td>Ca2+ increase; cell morphology, premature differentiation</td>
</tr>
<tr>
<td>9</td>
<td>50 Hz</td>
<td>L-type</td>
<td>Neural stem/progenitor cells</td>
<td>In vitro differentiation, neurogenesis</td>
</tr>
<tr>
<td>10</td>
<td>Static magnetic field</td>
<td>L-type</td>
<td>Rat</td>
<td>Reduction in edema formation</td>
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<tr>
<td>11</td>
<td>NMR</td>
<td>L-type</td>
<td>Tumor cells</td>
<td>Synergistic</td>
</tr>
<tr>
<td></td>
<td>Treatment</td>
<td>Cell Type</td>
<td>Biological Effect</td>
<td></td>
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<tr>
<td>11</td>
<td>NMR</td>
<td>L-type</td>
<td>Tumor cells</td>
<td>Synergistic effect of EMF on anti-tumor drug toxicity</td>
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<tr>
<td>12</td>
<td>Static magnetic field</td>
<td>L-type</td>
<td>Myelomonocytic U937 cells</td>
<td>Ca$^{2+}$ influx into cells and antiapoptotic effects</td>
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<tr>
<td>13</td>
<td>60 Hz</td>
<td>L-type</td>
<td>Mouse</td>
<td>Hyperalgesic response to exposure</td>
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<tr>
<td>14</td>
<td>Single nanosecond electric pulse</td>
<td>L-type</td>
<td>Bovine chromaffin cells</td>
<td>Very rapid increase in intracellular Ca$^{2+}$</td>
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<tr>
<td>15</td>
<td>Biphasic electric current</td>
<td>L-type</td>
<td>Human mesenchymal stromal cells</td>
<td>Osteoblast differentiation and cytokine production</td>
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<td>16</td>
<td>DC &amp; AC magnetic fields</td>
<td>L-type</td>
<td>ß-cells of pancreas, patch clamped</td>
<td>Ca$^{2+}$ flux into cells</td>
</tr>
<tr>
<td>17</td>
<td>50 Hz</td>
<td>L-type</td>
<td>Rat pituitary cells</td>
<td>Ca$^{2+}$ flux into cells</td>
</tr>
<tr>
<td>18</td>
<td>50 Hz</td>
<td>L-type, N-type</td>
<td>Human neuroblastoma IMR32 and rat pituitary GH3 cells</td>
<td>Anti-apoptotic activity</td>
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<tr>
<td>19</td>
<td>Nanosecond pulse</td>
<td>L-type, N-type, P/Q-type</td>
<td>Bovine chromaffin cells</td>
<td>Ca$^{2+}$ dynamics of cells</td>
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<tr>
<td>20</td>
<td>50 Hz</td>
<td>Not determined</td>
<td>Rat dorsal root ganglion cells</td>
<td>Firing frequency of</td>
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<tr>
<td></td>
<td>Very weak electrical fields</td>
<td>T-type</td>
<td>Sharks</td>
<td>Detection of very weak magnetic fields in the ocean</td>
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<tr>
<td>---</td>
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<tr>
<td>22</td>
<td>Short electric pulses</td>
<td>L-type</td>
<td>Human eye</td>
<td>Effect on electro-ocularogram</td>
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<tr>
<td>23</td>
<td>Weak static magnetic field</td>
<td>L-type</td>
<td>Rabbit</td>
<td>Baroreflex sensitivity</td>
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<tr>
<td>24</td>
<td>Weak electric fields</td>
<td>T-type</td>
<td>Neutrophils</td>
<td>Electrical and ion dynamics</td>
</tr>
<tr>
<td>25</td>
<td></td>
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</table>
The finding that EMF exposure acts via activation of VGCCs, provides for the first time, an answer to the puzzle of how exposure to EMFs composed of low energy photons can affect our biology and medicine. The effects of EMFs on the voltage across the plasma membrane can lead to partial depolarization and subsequent activation of VGCCs, leading to very rapid increases in intracellular Ca2+. Because increased intracellular Ca2+ can act, in turn, to stimulate NO synthesis, such NO increase may also have an important role.

Pilla recently showed that such EMF exposure can lead to almost instantaneous increases in both intracellular Ca2+ and also of NO synthesis (all occurring in less than 5 seconds):

Most responses physiological responses to Ca2+ and NO, act as follows: NO increasing levels of cGMP, leading in turn to stimulation of the cGMP-dependent protein kinase (protein kinase G).

In contrast, most pathophysiological effects of NO are mediated through its role as a precursor of peroxynitrite (ONOO-), leading to free radical generation and oxidative stress.

There are a series of therapeutic effects of EMFs, raising the question of how these might act. And there are a series of pathophysiologic effects of EMFs, raising the question of how these might act. I took what is probably the best documented example of each of these to determine apparent answers to these questions.

I found that the therapeutic effects of EMFs in stimulating bone growth, act via EMF stimulation of osteoblasts probably via NO, cGMP and increased protein kinase G.

I also found that the pathophysiologic effects of EMF exposure, inducing single strand breaks in cellular DNA, probably acts via increased NO, ONOO- and oxidative stress. Each of these results, then tend to confirm our preconceived notions of what mechanisms are likely to be involved!
What about EMF hypersensitivity (EHS)?? Anecdotal reports claim a number of similarities to MCS: These have high levels of co-morbity- that this they often occur together in the same patients. Physicians have reported that they both appear to respond to the same therapeutic approaches, approaches that I will argue may work by lowering the NO/ONOO- cycle. The symptoms of each vary quite a bit from patient to patient. Both appear to occur following previous exposure, chemical exposure in the case of MCS and EMF exposure in the case of EHS. The basic question that I am raising here is whether EHS is produced by the NO/ONOO- cycle and by long-term potentiation (LTP), as we think MCS is?

There is, in fact, a substantial literature showing that VGCC stimulation can lead to LTP, in much the same way that NMDA stimulation does. This is not surprising, given the fact that the downstream effects of VGCC stimulation are similar if not identical to those of NMDA stimulation.

A second question is whether VGCC elevation acts as part of NO/ONOO- cycle as does NMDA elevation? I argue here that VGCC elevation does act as part of the NO/ONOO- cycle, because lowered mitochondrial function/ATP levels lead to partial depolarization of the plasma membrane and therefore VGCC stimulation. Such VGCC stimulation, acts, in turn to increase intracellular Ca^{2+}, an important element of the cycle.
Therapy: How can we treat and hopefully cure NO/ONOO- cycle diseases? There are many agents that have been used to treat proposed NO/ONOO- cycle diseases that can be shown to lower cycle elements but we don’t have time to review this large literature here. Some of this is discussed in Chapter 15 of my book. But in general, there has not been any extensive study of combinations of agents aimed specifically at lowering the entire cycle and presumably this is what we need!

(PLEASE NOTE: I am a PhD, not an MD or ND and none of what I say here should be viewed as medical advice)

Let’s look again at the various parts of the cycle, as it has been proposed, to see why it is predicted to be so robust and what our challenges are in down-regulating the cycle.
It can be seen from the above, that the one element of the cycle that occurs in each of the five component cycles, shown above is peroxynitrite (ONOO-) and therefore a peroxynitrite scavenger may be expected to be particularly useful in treatment. One agent that is a powerful peroxynitrite scavenger is 5-methyltetrahydrofolate (5-MTHF) (see Rezk, FEBS Lett 2003;555:601–605; Antoniades, Circulation 2006;114:1193–1201). So, in principle, by using sufficient 5-MTHF, one should be able to be able to cure NO/ONOO- cycle disease. But it is more complicated than that. I have received information from four different sources, that most patients with the ME/CFS, MCS and fibromyalgia group of diseases, do not tolerate well doses above 300 micrograms per day of 5-MTHF. Why should that be?? When there is a lot of peroxynitrite present, particularly in the GI tract, then much of the 5-MTHF is oxidized presumably to the dihydro form, 5-MDHF which breaks down further to products that are lost from the folate pool. Presumably this leads not only to loss of reduced folates but also accumulation of some toxic product.
So how can we avoid both loss of 5-MTHF and accumulation of a toxic product of 5-MTHF oxidation? Most likely by using high doses of ascorbate (vitamin C). Ascorbate is both a peroxynitrite scavenger, although one needs high concentrations to be reasonably effective and scavenging peroxynitrite will, of course, lower 5-MTHF oxidation. Furthermore, high doses of ascorbate will reduce the 5-MDHF oxidation product back to 5-MTHF thus simultaneously lowering peroxynitrite mediated loss of 5-MTHF and greatly lowering accumulation of toxic oxidation products. In general by using high dose ascorbate along with substantial amounts of 5-MTHF, one should be able to much more effectively lower peroxynitrite than by using either one alone.

What dose of ascorbate should be used? If 5-MTHF is taken orally, then perhaps 1 to 2 g of oral ascorbate should be taken simultaneously. And as the tolerance of this combination becomes clear, it may be possible to repeat it two or three times per day.
One of the mechanisms that leads to mitochondrial dysfunction in the NO/ONOO-cycle is the massive stimulation of poly (ADP-ribose) polymerase (PARP) activity in the nucleus in response to DNA nicking by hydroxyl radical and other radical products of peroxynitrite, leading, in turn to a massive depletion of NAD/NADH in the cell. This depletion occurs because NAD is the substrate for this enzyme. And the SIRT1 enzyme is an NAD dependent deacetylase whose activity is strongly dependent in vivo on NAD levels in the cell. Consequently, it is essential to restore NAD levels before resveratrol can possibly be effective in treatment of and possibly cure of NO/ONOO- cycle diseases.

The best way to do this may be to use substantial doses of nicotinic acid, possibly using low flush niacin, to help restore NAD pools. It may also be useful to simultaneously use D-ribose, which is converted to PRPP which reacts enzymatically with nicotinic acid or nicotinamide to generate NMN and NAD. I don’t think one should use nicotinamide here for NAD generation because nicotinamide inhibits SIRT1 activity itself!

Let me just add one thing. I wonder whether Abram Hoffer’s treatment of schizophrenic patients with high dose nicotinic acid may have worked via increased SIRT1 activity.
Another promising agent is the resveratrol, a phenolic compound which acts via more than one pathway, but where a single pathway appears to be central to the important favorable effects for lowering the NO/ONOO- cycle:

Resveratrol $\rightarrow$ SIRT1 $\rightarrow$ lowered superoxide via at least five mechanisms (induction of all three superoxide dismutases, lowered NADPH oxidase, lowered mitochondrial superoxide generation), improved mitochondrial function, lowered NF-kappaB activity, lowered iNOS induction, increased BH4 production and consequent improved NOS coupling, lowered excessive NMDA activity (via two mechanisms), lowered peroxynitrite, lowered oxidative stress and lowered intracellular calcium levels. Essentially, the whole NO/ONOO- cycle is lowered by resveratrol raising SIRT1 activity!!

So is resveratrol the long awaited magic bullet to cure NO/ONOO- cycle diseases?? It probably is a good preventive agent, but curing such diseases is another matter. I suspect you knew that this was too good to be true, but why and how can we get around the limitations? What is the problem and how can we get around it?
Let’s go on to some other agents that are often used to treat proposed NO/ONOO-cycle diseases.

One of these is **magnesium**. Marginal or more severe magnesium deficiencies are common in many countries, due to the low magnesium levels in highly process foods and due to soil magnesium depletion due to intensive agriculture. Magnesium has a crucial role in regulating NMDA receptor activity due to the role of magnesium ions in blocking the channel of these receptors that can open to allow calcium influx. It follows from this that those with magnesium deficiencies are at great risk for generating NO/ONOO- cycle disease due to excessive NMDA activity.

Another agent used to treat these diseases is **fish oil** and similar lipids containing long chain omega-3 fatty acids **DHA and EPA**. These have anti-inflammatory activity, lowering the inflammatory effects of arachidonic acid-derived eicosanoids produced in excessive amounts when our diets have excessive omega-6 fatty acids, as is typical in most of our diets.

**Phospholipids** are also used to treat these diseases and may act, at least in part, by helping restore the oxidized cardiolipin the the inner membrane in the mitochondrion. It is possible that phosphatidyl serine may be particularly effective here, although we don’t know that, because there is a transporter that specifically transports phosphatidyl serine into the inner mitochondrial membrane.
**L-carnitine/acetyl-L-carnitine (ALC)** are other agents often used for treatment, with ALC being more active at least in part because it is transported more efficiently in the body. Until recently, I have assumed that the main mechanism of action of these compounds is to stimulate mitochondrial function, given the well established role of carnitine in fatty acid transport into mitochondria. However, recently, there has been established another mechanism that may turn out to be more important here, a mechanism that lowers excessive NMDA activity.

Glutamate is the main physiological agonist of the NMDA receptors and glutamate release stimulates not only the NMDA receptors but also the AMPA and kainate receptors and the metabotropic receptors. AMPA and kainate receptor stimulation produce still more NMDA receptor activity by depolarizing the plasma membrane, but the metabotropic receptor stimulation lowers the NMDA response. It has been shown that ALC/carnitine stimulate one of the metabotropic receptors, mGluR2, causing it to be more susceptible to glutamate stimulation, which lowers, in turn the response of the NMDA receptors.

However, as with many agents, ALC/carnitine may be a mixed blessing. Many NMDA antagonists lead to increased production of NMDA receptor when used chronically. Does this occur with ALC/carnitine?? Furthermore ALC/carnitine can produce at least a modest increase in NF-kappaB activity, so that could be a problem.
We discussed earlier the use of 5-MTHF together with high dose ascorbate to scavenge peroxynitrite. These should work effectively in an aqueous environment but not in the lipid phase of cells, where the acid form, peroxynitrous acid has substantial solubility. Carotenoids act however in the lipid phase as peroxynitrous acid scavengers.

When they do so, the cis-double bonds within carotenoids appear to have a special role, changing from cis to trans in the process. Natural carotenoids have some cis-double bonds. For example natural beta-carotene has roughly one cis-double bond per two molecules, whereas synthetic beta-carotene is essentially all trans. This may be important because most if not all clinical trials on beta-carotene have used synthetic beta-carotene. Other natural carotenoids, including lycopene and lutein/zeaxanthin, may have special roles in this process of peroxynitrous acid scavenging.

Agents that lower NF-kappaB activity include a number of chain breaking antioxidants, including phenolic and thiol antioxidants. It is unclear whether using these is adequate in lowering NF-kappaB activity, such that using herbal or pharmaceutical agents recognized to lower NF-kappaB activity via other mechanisms may also be important.
Sauna therapy has been used to treat several proposed NO/ONOO- cycle diseases. It has often been assumed to be acting via a detoxification mechanism. While some detoxification has been shown to occur of stored toxicants in the body, typically over a period of weeks, patients often report much more rapid symptomatic improvement. There is no published evidence, to my knowledge, showing that lowering of toxicants in the body is the main mechanism of symptomatic improvement.

I have argued that the main mechanism of symptomatic improvement in response to sauna therapy is produced by increased BH4 availability. The rate limiting enzyme in the de novo synthesis of BH4 is GTP cyclohydrolase I (GTPCH-I). This enzyme has been shown to be increased by two consequences of sauna therapy: induction of the heat shock protein Hsp90 and increased blood flow shear in the vasculature. And both of these lead to decreased nitric oxide synthase uncoupling which is produced by increased availability of BH4. Increased BH4 production in the heated regions of the body and in the vasculature should raise levels of circulating BH4, thus feeding tissues of the body with BH4 depletion, whether they are directly impacted by sauna treatment or not.

Sauna therapy via this mechanism may well be useful in the treatment of cases of many NO/ONOO- cycle diseases, whether these cases are characterized by elevated levels of toxicants in the body, or not.
Agents that raise the levels of reduced glutathione (GSH) in the body should be useful in the treatment of NO/ONOO− cycle diseases, lowering the oxidative stress that is one of the features of the cycle. It is common for glutathione, both reduced (GSH) and total glutathione (GSH + GSSG) to be depleted in tissues under oxidative stress. There are a number of agents that may be useful in helping restore GSH pools. These include a precursor of GSH de novo synthesis, N-acetylcysteine, α-lipoic acid, liposomal GSH or inhaled, nebulized or nasal spray GSH or oral acetylated GSH. It has been argued that sublingual GSH is also useful.

Agents that stimulate glutathione reductase (which uses NADPH to reduce GSSG to GSH), such as high dose riboflavin or niacin, may be useful and also possibly agents that increase the generation of NADPH via the pentose phosphate shunt, such as high dose thiamine may also be useful.
“Vitamin E” may be useful but may also be damaging, depending on the form and dosage used and the patient cohort studied. Synthetic (all rac) α-tocopherol, the usual form studied in clinical trials at high doses (400IU/day or more) induces an enzyme (CYP4F2) which degrades all the other forms of vitamin E including γ-tocopherol, δ-tocopherol, α-tocotrienol, β-tocotrienol, γ-tocotrienol and δ-tocotrienol. Consequently, high doses of α-tocopherol leads to a deficiency in all of these other forms of vitamin E. This might be OK if α-tocopherol had all of the activities of these other forms, but it is very clear that it does not.

γ&δ-tocopherol and tocotrienol all scavenge NO2 radical, an important breakdown product of peroxynitrite, but α-tocopherol does not. γ-tocopherol has important anti-inflammatory effects, acting to lower cyclooxygenase activity much more than α-tocopherol. Excitotoxicity caused by excessive NMDA activity works, in part via excessive activity of the 12-lipoxygenase enzyme; this enzyme is potently inhibited by α-tocotrienol which greatly lowers NMDA excitotoxicity but this property is not shown by α-tocopherol. γ&δ-tocotrienols have some anticancer properties with much higher activities than do the tocopherols. Tocotrienols have been shown to have higher antioxidant activities in membranes than do the similar tocopherols. Some cell types have been shown to have much higher transport activity, concentrating tocotrienols much more than tocopherols. And there is some evidence suggesting that tocotrienols may be more effective in protecting mitochondria than are tocopherols.
None of these observations negate important roles for $\alpha$-tocopherol. But they do suggest that when high dose synthetic $\alpha$-tocopherol is used in clinical trials, the accompanying loss of other forms of vitamin E is likely to have important negative consequences and may therefore be responsible for the many disappointing responses in such clinical trials.

In the context of the NO/ONOO- cycle, the roles of these other forms of vitamin E in scavenging NO2 radical, lowering inflammatory responses, lowering NMDA-induced excitotoxicity and in protecting mitochondrial activities are all reasons not to use such high dose synthetic $\alpha$-tocopherol in treatment of NO/ONOO- cycle diseases. It also suggests that use of 400 IU/day nutritional supplements of synthetic $\alpha$-tocopherol may make us more susceptible to some NO/ONOO- cycle diseases, rather than less susceptible. My own view, therefore, is that NO/ONOO- cycle diseases when treated with protocols using vitamin E, should be treated using modest doses of natural $\alpha$-tocopherol, much higher doses of $\gamma$-tocopherol and substantial doses of $\alpha$, $\gamma$, $\delta$-tocotrienols.
The last agent I wish to discuss is high-dose hydroxocobalamin form of vitamin B-12. Hydroxocobalamin when reduced in the body from the cobalt (III) form to the cobalt (II) form is a potent nitric oxide scavenger. It has also been recently reported to be both a superoxide scavenger and a peroxynitrite scavenger, but it is unclear to me whether these last two activities are likely to be physiologically important. Other forms of vitamin B-12 may also serve as precursors of hydroxocobalamin.

Ellis and Nasser published a placebo-controlled study showing efficacy of hydroxocobalamin IM injections (5 mg/twice a week) in ME/CFS-like patients back in 1973. Both IM and IV injections have been used clinically, as have hydroxocobalamin nasal spray and nebulized inhaled hydroxocobalamin. Oral hydroxocobalamin is probably of very limited value due to absorption being limited by the availability of intrinsic factor. Sublingual B-12 has been suggested to be useful, but increased sublingual absorption has not been confirmed in published studies, to my knowledge.

I think that hydroxocobalamin is likely to be a very useful, well tolerated agent for the treatment of NO/ONOO- cycle diseases.
In summary, we have some 21 chronic inflammatory diseases characterized by elevation of other elements of the NO/ONOO-cycle, most of which have a good fit to the five principles underlying the cycle.

None of these diseases can be cured and in most cases can even be effectively treated by conventional allopathic medicine. **It is my view, as a PhD biochemist, that naturopathic medicine is much better equipped to deal effectively with these diseases.** If this view is correct, you are in THE most important part of medicine. But in order to show that, I very desperately need your help! So you should take my talk also as a challenge – to join me to show that naturopathic medicine is where the action is in the treatment of chronic inflammatory disease.
High dose ascorbate can be viewed as a useful therapeutic agent in a different context – it can lower both sides of what is called the central couplet, as seen in Fig 2C. That is it can not only lower peroxynitrite, as discussed immediately above, but it can also lower loss of peroxynitrite mediated oxidation of BH4 by a second mechanism. When peroxynitrite oxidizes BH4, it produces BH3 which can be reduced back to BH4 by ascorbate – however again, one needs fairly high doses for this to be effective. So again if oral ascorbate is used, something on the order of 1 to 2 g doses may be needed. IV ascorbate can, of course, generate much higher levels and so may be still much more effective.

There is a third mechanism that may be useful but that is probably only going to contribute when high doses of IV ascorbate are used. Ascorbate being a reducing agent can reduce molecular oxygen to H2O2, which induces the rate limiting enzyme in BH4 de novo biosynthesis, GTP cyclohydrolase 1. This may act, then to increase de novo BH4 synthesis. H2O2 is of course an oxidant so here, one needs to be concerned about going too high, since oxidative stress has a major role in the NO/ONOO- cycle.
Summary:
1. 7 classes of chemicals implicated in MCS all act via excessive NMDA activity.
2. 6 other types of evidence also implicate excessive NMDA activity in MCS.
3. Genetics of susceptibility show that genes involved in chemical metabolism influence susceptibility to MCS.
4. #1-3 show beyond doubt that MCS is a real disease involving chemical exposure.
5. MCS is thought to be a NO/ONOO- cycle disease, with the cycle causing it to be chronic and with chemicals acting to initiate or elevate the cycle through their action raising NMDA activity.
6. Long-term potentiation (LTP) also has a probable role in MCS in the brain and possibly in some other tissues.

7. Other chronic, possible NO/ONOO- cycle diseases may be initiated by chemicals acting via excessive NMDA activity but also by other stressors acting in various ways.

8. EMF exposure acts by activating voltage-gated calcium channels (VGCCs) leading in turn to increased intracellular Ca2+ and NO.

9. These may act, in turn, to produce electromagnetic hypersensitivity (EHS) via the NO/ONOO- cycle and also LTP (similar mechanisms to MCS).

10. The major goal in treatment is to lower the NO/ONOO- cycle.

11. The robust nature of the NO/ONOO- cycle makes this a major challenge.