

NMDA sensitization and stimulation by peroxyxynitrite, nitric oxide, and organic solvents as the mechanism of chemical sensitivity in multiple chemical sensitivity

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ABSTRACT Multiple chemical sensitivity (MCS) is a condition where previous exposure to hydrophobic organic solvents or pesticides appears to render people hypersensitive to a wide range of chemicals, including organic solvents. The hypersensitivity is often exquisite, with MCS individuals showing sensitivity that appears to be at least two orders of magnitude greater than that of normal individuals. This paper presents a plausible set of interacting mechanisms to explain such heightened sensitivity. It is based on two earlier theories of MCS: the elevated nitric oxide/peroxyxynitrite theory and the neural sensitization theory. It is also based on evidence implicating excessive NMDA activity in MCS. Four sensitization mechanisms are proposed to act synergistically, each based on known physiological mechanisms: Nitric oxide-mediated stimulation of neurotransmitter (glutamate) release; peroxyxynitrite-mediated ATP depletion and consequent hypersensitivity of NMDA receptors; peroxyxynitrite-mediated increased permeability of the blood-brain barrier, producing increased accessibility of organic chemicals to the central nervous system; and nitric oxide inhibition of cytochrome P450 metabolism. Evidence for each of these mechanisms, which may also be involved in Parkinson's disease, is reviewed. These interacting mechanisms provide explanations for diverse aspects of MCS and a framework for hypothesis-driven MCS research.—Pall, M. L. NMDA sensitization and stimulation by peroxyxynitrite, nitric oxide, and organic solvents as the mechanism of chemical sensitivity in multiple chemical sensitivity. *FASEB J.* 16, 1407–1417 (2002)

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MULTIPLE CHEMICAL SENSITIVITY (MCS) is a condition in which many cases appear to be preceded by and putatively induced by exposure to various organic solvents or certain pesticides, notably organophosphates or carbamates (1–7). After such exposure, MCS sufferers report being hypersensitive to a wide range of organic chemicals, including hydrophobic, volatile organic solvents. There is no accepted etiologic mechanism for MCS, but it appears not to be centered on an IgE-based allergic mechanism (1). Epidemiological

studies report that MCS is surprisingly common in the U.S. In reviewing such epidemiological studies of MCS, Sorg concluded that the “prevalence of severe MCS in the U.S. is ~4% with greatly reduced quality of life for the patient” (2). She states (2) that “Less severe problems with chemical exposures have been reported in ~15–30% of the population.” Similar estimates of MCS prevalence were obtained in two recent studies (8, 9).

The reported chemical sensitivity of many MCS people is exquisite. For example, MCS patients often report being sensitive to perfumes worn by people who may be seated several seats away or may be walking by them, whereas the perfume wearers appear to suffer no obvious sensitivity (1, 5, 7). From such anecdotal reports, it may be suggested that MCS patients may suffer from chemical sensitivity two or more orders of magnitude greater than do normal individuals. This suggestion of at least two orders of magnitude of unusual chemical sensitivity was explicitly proposed by Cullen (3) as a diagnostic feature for MCS, writing that “Exposures that elicit symptoms must be very low, by which we mean many standard deviations below ‘average’ exposures known to cause adverse human responses. Since data on the range of ‘normal’ responses are often unavailable, a rule of thumb would be that exposures are known to be generally lower than 1% of established threshold values (TLVs).” Such MCS sensitivity to concentrations two or more orders of magnitude lower than those producing similar sensitivity responses in normal controls was reported in laboratory studies by Fiedler and Kipen of phenyl ethyl alcohol sensitivity (10).

Probably the most serious deficiency of the MCS literature is that there is no plausible mechanism by which previous chemical exposure can induce such an exquisite sensitivity to a wide range of chemicals. For example, one of the MCS skeptics, R. E. Gots states that (11) “There is no known mechanism whereby low levels of chemical or chemicals of widely varied chemical structure can interact adversely with numerous organ systems. Even the theories advanced by clinicians arguing intensely for an organic etiology fail to do this.”

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Diverse hydrophobic compounds can interact with biological response mechanisms simply by binding to a hydrophobic pocket, as one might postulate for a known bacterial chemotaxis system that responds to a wide variety of hydrophobic compounds (12). Similarly, Sharom et al. (13) have described the P-glycoprotein as a “hydrophobic vacuum cleaner” binding to a wide variety of hydrophobic compounds, so the issue of common response to various hydrophobic chemicals can be laid to rest. However, the need for a plausible mechanism for inducing such exquisite chemical sensitivity in humans, as reported in MCS, remains. Antelman (14) describes similar responses to a wide variety of chemical stressors in an animal model of MCS, suggesting that a neural sensitization mechanism is involved but leaving open the issue of how the high-level sensitivity reported in MCS can be explained. There appears to be agreement among certain proponents of a physiological basis for MCS, as well as one skeptic, that a new paradigm is needed to explain any such physiological mechanism (11, 15–17). This paper proposes a plausible set of mechanisms for induced exquisite chemical sensitivity based on two previously proposed theories of MCS: the elevated nitric oxide/peroxynitrite theory and the neural sensitization theory. It may be viewed as a fusion of and extension of those theories. The mechanisms proposed are supported by evidence for these two earlier theories as well as by other evidence, as discussed below.

THE ELEVATED NITRIC OXIDE/PEROXYNITRITE AND THE NEURAL SENSITIZATION THEORIES OF MCS

MCS has multiple overlaps with three other medical conditions: chronic fatigue syndrome (CFS), fibromyalgia (FM), and post-traumatic stress disorder (PTSD). The four conditions share overlapping symptoms; many people are diagnosed as having more than one of these disorders. Many cases appear to be preceded by a short-term stress, only to be followed by a chronic condition that typically lasts for years or decades. Gulf War syndrome can be viewed as a combination of all four (18–22). The overlaps among these four conditions have led several research groups to propose they may share a common etiologic mechanism (16, 23–29). To my knowledge, the only such overall common mechanism proposed for all four is the elevated nitric oxide/peroxynitrite mechanism (28–33). There is evidence that the short-term stress for each—most commonly infection in the case of CFS, chemical exposure in the case of MCS, physical trauma in the case of FM, and severe psychological stress in the case of PTSD—produces increases in nitric oxide (28–32). Nitric oxide reacts with superoxide to form the potent oxidant peroxynitrite. According to this theory, peroxynitrite can act through six different positive feedback loop mechanisms to increase the levels of nitric oxide and its other precursor, superoxide, which in turn react to

form more peroxynitrite (30). In this way, a biochemical vicious cycle is initiated and maintained that produces the chronic nature of these four conditions. Various types of evidence supporting this proposed mechanism have been reviewed earlier (28–33). In the case of MCS, 10 types of supportive evidence are summarized in **Table 1**.

The second precursor theory to this paper is the neural sensitization theory originally proposed by Bell and co-workers (34) and since adopted with various modifications by several laboratories (2, 14, 34–39). This theory is based on the evidence for nervous system dysfunction in MCS and the fact that the central nervous system (CNS), especially the limbic system, can be shown to produce increased long-term synaptic sensitivity induced by previous electrical and chemical stimulation. The mechanism here is thought to be that

TABLE 1. *Types of evidence implicating nitric oxide/peroxynitrite in MCS^a*

1. Several organic solvents thought to be able to induce MCS, formaldehyde, benzene, carbon tetrachloride, and certain organochlorine pesticides all induce increases in nitric oxide levels.
2. A sequence of action of organophosphate and carbamate insecticides is suggested whereby they may induce MCS by inactivating acetylcholinesterase and thus produce increased stimulation of muscarinic receptors, which are known to produce increases in nitric oxide.
3. Evidence for induction of inflammatory cytokines by organic solvents that induce the inducible nitric oxide synthase (iNOS). Elevated cytokines are an integral part of the proposed feedback mechanism of the elevated nitric oxide/peroxynitrite theory (28).
4. Neopterin, a marker of the induction of the iNOS, is reported to be elevated in MCS.
5. Increased oxidative stress has been reported in MCS; antioxidant therapy may produce improvements in symptoms, as expected if the levels of oxidant peroxynitrite are elevated.
6. In a series of studies of a mouse model of MCS involving partial kindling and kindling, excessive NMDA activity and nitric oxide synthesis were shown to be required to produce the characteristic biological response.
7. The symptoms exacerbated on chemical exposure are similar to the chronic symptoms of CFS (1); these may be explained by several known properties of nitric oxide, peroxynitrite, and inflammatory cytokines, each of which have a role in the proposed mechanism (30).
8. These conditions (CFS, MCS, FM, and PTSD) are often treated through intramuscular injections of vitamin B-12; B-12 in the form of hydroxocobalamin is a potent nitric oxide scavenger in vitro and in vivo (29).
9. As discussed below, peroxynitrite is known to induce increased permeabilization of the blood-brain barrier; such increased permeabilization is reported in a rat model of MCS.
10. Five types of evidence implicate excessive NMDA activity in MCS, an activity known to increase nitric oxide and peroxynitrite levels (see text below).

^a Evidence for this list is discussed in ref 26 except as indicated by specific references listed in the table.

of long-term potentiation (LTP), a central mechanism for learning and memory where the synapses show long-term increases in sensitivity to stimulation. CNS dysfunction in MCS has been confirmed by SPECT (40, 41) and PET scans (42, 43) of the brains of MCS patients as well as changes in EEG patterns (44–47). Ashford and Miller list 10 different similarities between time-dependent neural sensitization and MCS (pp. 258–259, ref 1).

As discussed below, nitric oxide and the NMDA excitatory neurotransmission system are implicated in LTP, the presumed mechanism of neural sensitization. Not surprisingly, then, several animal models of MCS implicate excessive NMDA activity. For example, in the recent New York Academy of Sciences volume on neural sensitization and MCS, three animal models discussed implicate excessive NMDA activity (48–50) and a fourth (51), involved sensitization to formaldehyde and cocaine, two agents known to stimulate NMDA activity (52–59). Studying a mouse model of MCS, Itzhak and co-workers (52, 53, 60, 61) have demonstrated an essential role for excessive NMDA activity and nitric oxide synthesis in producing the physiological responses in that model.

When stimulated, the NMDA system has been found to produce increases in nitric oxide through a mechanism involving increased Ca^{2+} influx into the cell and consequent increased intracellular Ca^{2+} levels, stimulating the calcium-dependent nitric oxide synthases nNOS and eNOS. Multiple research groups have reported that excessive NMDA stimulation produces increases in nitric oxide and its oxidant product peroxynitrite (62–65). Excessive NMDA activity leading to excessive levels of nitric oxide and peroxynitrite has been implicated in several neurodegenerative diseases including amyotrophic lateral sclerosis, Parkinson's disease, AIDS-related dementia, stroke, epilepsy, Huntington's disease, and Alzheimer's disease (67–72). As discussed in the next section, nitric oxide and peroxynitrite may act through known mechanisms to increase both the stimulation of and the sensitivity of NMDA receptors.

NMDA/NITRIC OXIDE/PEROXYNITRITE MECHANISM FOR CHEMICAL SENSITIVITY

For this section, I am asking the reader to take as a working hypothesis that volatile organic solvents act in MCS by increasing NMDA activity. The evidence for this (discussed below) is suggestive but not conclusive. If one assumes this action of organic solvents, it follows that volatile organic solvent exposure, by producing excessive NMDA activity, can produce a consequent increase in nitric oxide and peroxynitrite (62–66), as discussed above. It is proposed here that the central mechanism of MCS is based on widespread stimulation of NMDA activity in the limbic system areas of the CNS induced by organic solvents, with widespread increases in nitric oxide and peroxynitrite. The neural sensitiza-

tion is based on the finding that the consequent nitric oxide can increase the stimulation of NMDA receptors whereas the resulting peroxynitrite can increase the sensitivity of those receptors to such stimulation (Fig. 1).

The background for this is the mechanism of LTP. LTP is known to involve presynaptic and postsynaptic changes, producing increased synaptic transmission (72–77). Both are known to be dependent on increases in NMDA activity and the consequent increases in postsynaptic Ca^{2+} concentrations (72–77). The presynaptic changes are dependent on the action of one or more retrograde messengers that diffuse from the postsynaptic cell to the presynaptic cell and produce changes in the presynaptic cell, producing in turn increased release of excitatory neurotransmitter (glutamate). Nitric oxide is known to act as a retrograde messenger in long-term potentiation (77–85). Accordingly, if organic solvent exposure acts to induce excessive NMDA stimulation, the subsequent nitric oxide increase may produce inappropriately high glutamate release, leading to inappropriately high NMDA and non-NMDA (AMPA, kainate, and metabotropic) receptor activity (Fig. 1). The focus here is primarily on excessive stimulation of the NMDA receptors.

The peroxynitrite produced by excessive of NMDA activity may play a role in a second mechanism, acting on postsynaptic cells containing NMDA receptors. It is known that peroxynitrite can deplete ATP pools by two major mechanisms. It attacks proteins in mitochondria, leading to lessened ATP generation (reviewed in refs 86, 87). It also acts to nick DNA, thus activating the poly (ADP-ribose) polymerase, leading to depletion of the NAD pool and, because NAD/NADH have essential roles in oxidative ATP generation, to ATP depletion as well (88–90). It is well known that when neurons containing NMDA receptors have lowered ATP pools, their NMDA receptors become hypersensitive to stim-

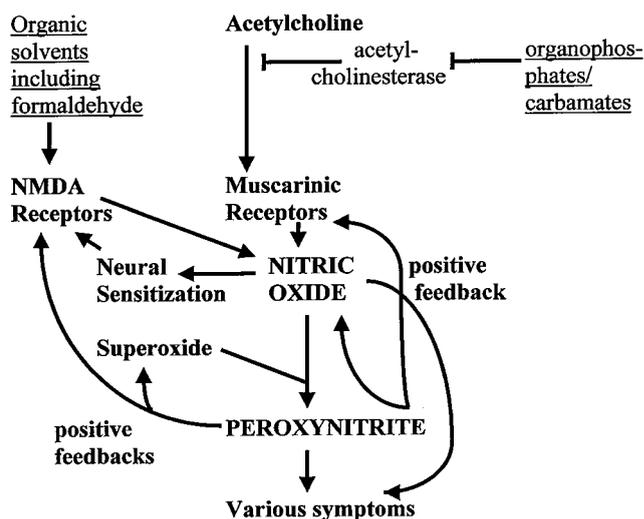


Figure 1. MCS model. The proposed role of organic solvents is diagrammed from the top left and the proposed role of organophosphates and carbamates is diagrammed from the top right. Arrows indicate stimulatory interactions.

ulation (Fig. 1) due to lowering of the plasma membrane potential (91–96). The NMDA stimulation produced by ATP depletion will be expected to produce an immediate increase in NMDA activity and stimulate NMDA-dependent LTP, thus producing longer term increases in synaptic activity.

The combination of nitric oxide-mediated increased neurotransmitter release and peroxynitrite-mediated increased sensitivity of the NMDA receptors, acting synergistically, is proposed to be the central mechanism of chemical sensitivity in MCS. When NMDA receptors are hyperactive, they produce increases in nitric oxide and peroxynitrite, exacerbating the neurotransmitter release and NMDA sensitization. Thus, it may be argued that within the vicious cycle mechanism maintaining elevated nitric oxide and peroxynitrite, as proposed in the elevated nitric oxide/peroxynitrite theory, there is another potential vicious cycle mechanism limited to regions of the nervous system containing NMDA receptors: excessive NMDA activity produces increased levels of nitric oxide and peroxynitrite, which in turn act to both activate and sensitize the NMDA receptors (Fig. 1).

The two mechanisms proposed above for long-term NMDA hypersensitivity/hyperactivity in MCS do not rule out other such mechanisms. It is known that the mechanisms involved in LTP are quite complex (74–76). In addition, MCS individuals are often reported to have low Mg^{2+} pools (7), and Mg^{2+} is known to lower NMDA sensitivity (91).

PESTICIDES AND OTHER FACTORS IN MCS

Besides the role of volatile organic solvents in initiating cases of MCS, other cases appear to be initiated through the action of pesticides, particularly organophosphate and carbamate pesticides. These were proposed earlier to initiate sensitivity via a mechanism different from NMDA stimulation, one that is also expected to produce increases in nitric oxide and peroxynitrite (28): Organophosphates and carbamates are known inhibitors of acetylcholinesterase, producing elevated levels of acetylcholine. Acetylcholine will activate the muscarinic receptors, which are known to produce increases in nitric oxide (28 and Fig. 1, top right). In this way, excessive muscarinic activity may initiate the same biochemical consequences as excessive NMDA activity.

Miller and Mitzel (6) studied cases of MCS that appeared to have been induced by organophosphate exposure and compared them with other cases that appeared to have been induced by organic solvent exposure. The latter cases were from people in buildings that had been remodeled (“sick building syndrome”), thus exposing the individuals to outgassing of organic solvents from the building materials. They reported striking similarities in symptoms of the two groups (6), suggesting that MCS mechanisms are likely

to be similar. The severity of symptoms was somewhat greater in the pesticide exposed cases, consistent with a separate pathway of action for organophosphate pesticides, as suggested here.

In a primate (97) and two rodent models (98, 99) of acute organophosphate toxicity as well as in humans (100), acute symptoms are treated not only with a muscarinic antagonist, but also with an NMDA antagonist, suggesting that excessive muscarinic stimulation will lead indirectly to excessive NMDA activity, as suggested by Fig. 1.

A study of Gulf War veterans specifically implicated an organophosphate in the induction of chronic neurological symptoms because of the association of a specific genetic polymorphism of a gene encoding the enzyme PON1, which metabolizes organophosphates with the occurrence of such symptoms (101, 102).

It was noted before that there are multiple overlaps among MCS, CFS, FM, and PTSD, with many individuals being diagnosed with more than one. There is considerable evidence that stressors commonly preceding cases of these other three conditions (infection, physical trauma, and severe psychological stress) may act to increase nitric oxide levels (29, 30, 32). It seems possible, therefore, that these individual stressors may be initiating factors for more than one of these conditions. Accordingly, Bell et al. (103) have reported that psychological trauma may be a risk factor for MCS. The possible role of other stressors may also help explain the observation that substantial numbers of MCS patients cannot identify a specific chemical exposure preceding the onset of their MCS symptoms (104). Exposure to carbon monoxide may help explain cases where there is no identifiable initiating exposure. Carbon monoxide has been proposed by Donnay (105, 106) to be a possible initiating chemical in MCS; because it is odorless and tasteless, carbon monoxide exposure may not be apparent to its victims. Carbon monoxide exposure can lead to chronic sequelae (29), with symptoms similar to those in CFS and MCS, and carbon monoxide is reported to induce excessive production of the two precursors of peroxynitrite: superoxide and nitric oxide (29). Several factors may influence individual susceptibility to these conditions, including genetic, hormonal, nutritional, and other factors (32). Factors producing variable individual susceptibility may explain the observations that in sick building syndrome situations, some individuals may develop MCS but others do not despite presumably similar chemical exposure.

TWO ACCESSORY MECHANISMS IN MCS: INCREASED BLOOD–BRAIN BARRIER PERMEABILITY AND DECREASED CYTOCHROME P450 ACTIVITY

Nitric oxide and peroxynitrite may act via two other known mechanisms, both of which may be expected to

increase chemical sensitivity. Peroxynitrite was proposed earlier (28) to increase the permeability of the blood–brain barrier in MCS, based on four studies that reported such peroxynitrite-mediated permeabilization in certain diseases (107–110). In the 15 months since that proposal was made, eight other reports have been published, greatly strengthening the evidence that peroxynitrite increases permeability of the blood–brain barrier (111–118). Such permeabilization will be expected of course to increase chemical access to the CNS and thus increase chemical sensitivity generated in the CNS. Increased blood–brain barrier permeability in response to chemical exposure has been reported by Abou-Donia and co-workers in a rat model of MCS (119). Evidence suggesting such increased permeability has been reviewed for the related conditions of PTSD (29) and CFS (120).

Nitric oxide is a known inhibitor of cytochrome P450 metabolism (121–124), as might be expected given the binding of nitric oxide to protein heme groups. Because cytochrome P450s are widely involved in the metabolism of hydrophobic molecules, such P450 inhibition will slow the metabolism of these compounds and cause them to be found in higher levels in the body.

These four mechanisms proposed to be involved in MCS are summarized in **Table 2**. Of these, two involve nitric oxide and two involve peroxynitrite. The first two mechanisms listed in Table 2 are closely related to neural sensitization theory of MCS and all four are closely related to the elevated nitric oxide/peroxynitrite theory.

EVIDENCE FOR EXCESSIVE NMDA ACTIVITY IN MCS AND ORGANIC SOLVENT STIMULATION OF NMDA ACTIVITY

Four types of evidence suggest excessive NMDA activity in MCS; two additional types suggest that organic solvents may act by stimulating NMDA activity.

There is evidence suggesting that excessive NMDA activity is involved in other members of this overlapping group of medical conditions. The evidence implicating

TABLE 2. Four mechanism by which nitric oxide or peroxynitrite may act to increase chemical sensitivity at NMDA receptors

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- Nitric oxide acts as a retrograde messenger, stimulating the presynaptic cells to become more active in releasing neurotransmitter (glutamate)
 - Peroxynitrite depletes ATP pools via two different mechanisms, causing NMDA receptors in depleted cells to be hypersensitive to stimulation
 - Peroxynitrite increases permeability of the blood–brain barrier, increasing accessibility of organic chemicals to the central nervous system
 - Nitric oxide inhibits cytochrome P450 metabolism of organic compounds, producing increased levels of organic compounds that may stimulate NMDA activity
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excessive NMDA activity in FM and PTSD has been reviewed recently (29). This suggests that excessive NMDA activity may have a role in MCS as well. The evidence for such excessive NMDA activity in various animal models of MCS was discussed above. To the extent that such animal models mirror the mechanism of MCS in humans, these observations may suggest a role in the human condition.

Miller and Mitzel (6) reported that many MCS patients appear to be hypersensitive to monosodium glutamate, a potential excitotoxin that can act to stimulate NMDA receptors (64, 66, 91).

A recent genetic study may provide a third link between MCS and NMDA activity. It was reported that an allele encoding the CCK-B receptor was significantly associated with increased MCS prevalence (125). It is known that the CCK-B receptor modulates NMDA activity (126, 127), providing another, albeit indirect linkage, between NMDA receptor activity and MCS. These and other interactions between the CCK-B receptor and NMDA activity have been reviewed by Adamec (128).

Do organic chemicals act to stimulate NMDA activity? One organic chemical often implicated in MCS—formaldehyde—clearly does so. Such NMDA stimulation by formaldehyde was first demonstrated by groups in Britain and the U.S. (129, 130) studying the mechanism of pain generation by formaldehyde and has been confirmed by recent studies (see, for example, refs 55–59). In these studies, NMDA antagonists and nitric oxide synthase inhibitors lower pain responses to formaldehyde exposure, showing a role for excessive NMDA activity and excessive nitric oxide synthesis in producing the pain responses. These studies clearly demonstrate that one organic solvent can act via NMDA stimulation. They also demonstrate that pain, which is a common symptom of MCS (as well as CFS, FM, and PTSD) can be produced by excessive NMDA activity and consequent elevated nitric oxide levels.

Organic solvent action in MCS was first suggested to act via NMDA activity by Donald Dudley based on clinical observations on his MCS patients (131). Dudley observed that the known NMDA antagonist, dextromethorphan, appeared to block the effects of organic solvents on his MCS patients. He prescribed dextromethorphan for his MCS patients on an episodic basis, suggesting they take it when unavoidably exposed to organic solvents or other triggers of MCS symptoms, reporting that the drug appeared to block or reverse the development of symptoms. In an interview (<http://members.aol.com/DonationDrive/BrainMapping.html>), Dudley described the effect of dextromethorphan as follows: “As a treatment, blockers of glutamate and aspartate are used to stop the reaction before it gets started. Prior to treatment, a patient would walk by someone with perfume on and their whole day would be ruined, they’re practically unable to think. With treatment, there is no reaction at all.”

Two other physicians in Washington state, Gordon

Baker and David Buscher, have prescribed dextromethorphan for their MCS patients in a similar fashion, with apparently similar results (G. Baker and D. Buscher, personal communications). I have talked with two MCS patients who have used dextromethorphan on an episodic basis after unavoidable chemical exposure, and both observed that most of their symptoms were abrogated by the drug.

Dudley assumed that organic solvents act by directly stimulating NMDA receptor activity (131), but there is no evidence supporting such a direct interaction. However, given the complexities of the NMDA system and its interaction with other regulatory mechanisms, there are many possible indirect targets for the action of organic solvents that may lead to organic solvent-mediated NMDA stimulation. Three possible indirect mechanisms are suggested here, two involving proposed increased nitric oxide synthesis, leading to NMDA hyperactivity, as suggested above. The third involves a different, mitochondrial mechanism.

1) It was suggested above that an attractive mechanism for the action of a wide variety of hydrophobic compounds in inducing MCS may be for them to bind to a hydrophobic pocket in some critical protein. Such a hydrophobic pocket might have little specificity of binding except for the requirement for hydrophobicity. The protein PIN appears to be just such a protein. PIN is a small protein that was discovered as an inhibitor of the nNOS isozyme (132), the major enzyme synthesizing nitric oxide in brain. PIN forms a dimer containing what is described as "a hydrophobic groove" into which a 13 residue segment of nNOS binds (133, 134), producing enzyme inhibition. Clearly, if binding of hydrophobic solvent molecules in that groove inhibits such binding and stimulates nNOS activity, this will be expected to lead to substantial increases in nitric oxide synthesis in brain, which would be expected to stimulate NMDA activity, as described above.

2) A second possible mechanism involves the reported stimulation of calcium channels by organic solvents (135–138). Because the nNOS and eNOS nitric oxide synthase isozymes are both Ca^{2+} -dependent enzymes, calcium channel stimulation will stimulate their activity, thus increasing nitric oxide synthesis, which is proposed to stimulate NMDA activity.

3) Many hydrophobic solvents are thought to act by disrupting mitochondrial membrane structure and function, uncoupling oxidative phosphorylation (139–142) while increasing the generation of superoxide radical, the other precursor of peroxynitrite (with nitric oxide) (for a recent review, see ref 139). This response appears to produce a prolonged generation of free radicals and other oxidants in brain (143). Given the known action of other agents that disrupt mitochondrial ATP generation in stimulating NMDA activity (91–96) these actions of a wide array of organic solvents may be an attractive mode of action in inducing NMDA hyperactivity and MCS.

Each type of evidence discussed in this section should be viewed as suggestive but not convincing. For exam-

ple, the animal model data may always be questioned as to relevance of the animal model to the human condition. Formaldehyde may act by stimulating NMDA activity, but that does not show that other organic solvents do likewise. The dextromethorphan data are based on clinical observations that have not even been published in any peer-reviewed form. The three specific possible targets for organic solvent action are suggestive, but there is no evidence implicating any of these targets in MCS. Part of the goal here is to suggest potentially important foci for hypothesis-driven research, and clearly the many uncertainties in this part of the hypothesis suggest many such topics of future study.

MULTIORGAN DYSFUNCTION IN MCS: POSSIBLE MECHANISMS

More symptoms of MCS may be attributed to CNS dysfunction than may be attributed to dysfunction of any other organ or organ system. However, there are MCS symptoms that are not attributed to a CNS origin. In her MCS review, Sorg (ref 2, Table 1) lists 18 neurological symptoms as being found in MCS but lists other symptoms of cardiovascular (two), respiratory (seven), gastrointestinal (three), genitourinary (four), musculoskeletal (five), and dermatologic (one) origin. Thus, many of the MCS symptoms appear to be of neurological origin but various other organs appear to be affected in many cases. NMDA receptors are widely distributed in the central and peripheral nervous system, but other cell types are not known to carry such receptors. Whereas mechanisms such as nNOS and eNOS control or mitochondrial dysfunction will affect a variety of tissues, three of the four sensitivity mechanisms described in Table 2 should primarily affect the CNS. It seems reasonable, therefore, to consider mechanisms by which central neural sensitization and CNS nitric oxide/peroxynitrite elevation may affect various tissues.

Nitric oxide and peroxynitrite are thought to have short half-lives *in vivo* on the order of 1 s, limiting their spread from their tissue of origin. However, inflammatory cytokines are proposed to be part of the mechanism leading to increased nitric oxide (30); such cytokines will circulate to a variety of tissues and have been reported to be induced by organic solvents (28). Furthermore, nitric oxide itself has recently been shown to bind to heme groups of hemoglobin and, when stabilized by such binding, can circulate to various regions of the body where it can be pumped out into the tissues (144). Thus, inflammatory cytokine elevation and nitric oxide transport may be mechanisms by which elevated nitric oxide in one tissue (i.e., CNS) may lead to spreading to other tissues.

Several other mechanisms to spread beyond the CNS have been discussed elsewhere, including autonomic dysfunction, Meggs' neurogenic inflammation hypoth-

esis, and neuroendocrine including HPA axis dysfunction (4, 35, 145–147).

DO ORGANIC SOLVENTS ACT BY STIMULATING NMDA ACTIVITY IN INDUCING PARKINSON'S DISEASE?

Several epidemiological studies have established that organic solvent exposure is an important risk factor in Parkinson's disease (148–153). These results suggest but do not prove that organic chemicals may induce Parkinson's disease. However there is no known mechanism by which such organic solvents may act to induce Parkinson's disease. Excessive NMDA activity is associated with Parkinson's disease in animal models (95, 96, 154) and humans (155–162). The drug MPTP, which is known to induce a Parkinson's disease-like response in humans and various animal models, acts by inhibiting mitochondrial function and consequent ATP generation, which acts in turn to activate NMDA receptor activity (95). These observations raise the question as to whether organic solvents may act, as proposed above for MCS, to stimulate NMDA hyperactivity specifically in the substantia nigra, the tissue primarily affected in Parkinson's disease, providing an explanation for their association with Parkinson's disease.

SUMMATION

Epidemiological studies report that the prevalence of MCS is roughly similar to that of diabetes and glucose intolerance. However, the funding for MCS research is probably less than a thousandth of that available for diabetes research. There is a critical need for clear and compelling hypotheses, consistent with the many reported properties of MCS, to provide a basis for future research. The current hypothesis has some important strong points and weak points. Its strong points are that given the support previously cited for the elevated nitric oxide/peroxynitrite theory and the neural sensitization theory, it makes sense to put them together to determine whether they complement each other. When those two theories and known properties of LTP and the NMDA receptor system are put together, they lead us to the current hypothesis. The four proposed consequent mechanisms (nitric oxide-mediated stimulation of neural transmitter release, peroxynitrite-mediated stimulation of postsynaptic NMDA sensitization, peroxynitrite-mediated blood–brain barrier permeabilization, and nitric oxide inhibition of cytochrome P450 metabolism) are all expected to act synergistically, producing the type of exquisite sensitivity reported in MCS. These four mechanisms are individually well documented, but their possible roles in MCS have not been adequately studied. The weak point of the current hypothesis is clearly the evidence supporting the inference that volatile organic solvents act to stimulate NMDA activity in MCS. It is the goal here to encourage

hypothesis-driven research to test the strong and the weak points of this hypothesis of CFS etiology. **[F]**

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