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## Potential animal model of multiple chemical sensitivity with cholinergic supersensitivity

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### Abstract

Multiple Chemical Sensitivity (MCS) is a clinical phenomenon in which individuals, after acute or intermittent exposure to one or more chemicals, commonly organophosphate pesticides (OPs), become overly sensitive to a wide variety of chemically-unrelated compounds, which can include ethanol, caffeine and other psychotropic drugs. The Flinders Sensitive Line (FSL) rats were selectively bred to be more sensitive to the OP diisopropylfluorophosphate (DFP) compared to their control counterparts, the Flinders Resistant Line (FRL) rats. The present paper will summarize evidence which indicates that the FSL rats exhibit certain similarities to individuals with MCS. In addition to their greater sensitivity to DFP, the FSL rats are more sensitive to nicotine and the muscarinic agonists arecoline and oxotremorine, suggesting that the number of cholinergic receptors may be increased, a conclusion now supported by biochemical evidence. The FSL rats have also been found to exhibit enhanced responses to a variety of other drugs, including the serotonin agonists *m*-chlorophenylpiperazine and 8-OH-DPAT, the dopamine antagonist raclopride, the benzodiazepine diazepam, and ethanol. MCS patients report enhanced responses to many of these drugs, indicating some parallels between FSL rats and MCS patients. The FSL rats also exhibit reduced activity and appetite and increased REM sleep relative to their FRL controls. Because these behavioral features and the enhanced cholinergic responses are also observed in human depressives, the FSL rats have been proposed as a genetic animal model of depression. It has also been reported that MCS patients have a greater incidence of depression, both before and after onset of their chemical sensitivities, so cholinergic supersensitivity may be a state predisposing individuals to depressive disorders and/or MCS. Further exploration of the commonalties and differences between MCS patients, human depressives, and FSL rats will help to elucidate the mechanisms underlying MCS and could lead to diagnostic approaches and treatments beneficial to MCS patients.

**Keywords:** Animal model of MCS; Organophosphate DFP; FSL rats; Human depressives; Cholinergic supersensitivity

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## 1. Introduction

Multiple Chemical Sensitivity (MCS) is a clinical phenomenon in which individuals, after acute or intermittent exposures to one or more chemicals, commonly organophosphate pesticides (OPs), report that they become overly sensitive to a wide variety of chemically-unrelated compounds, which can include ethanol, caffeine and other psychotropic drugs (Cullen, 1987; Ashford and Miller, 1989, 1991; Bell et al., 1992; Miller, 1994). Symptoms commonly reported by these individuals include fatigue, cognitive difficulties, depression, irritability, headaches, dyspnea, digestive problems, musculoskeletal pain, and numbness in their extremities. Symptoms often overlap those of better recognized medical illnesses such as depression, somatization disorder, chronic fatigue syndrome, fibromyalgia, asthma and others. However, a distinguishing feature of MCS is the patients' assertion that their symptoms are triggered by common exposures to low levels of volatile organic chemicals (for example, fragrances, insecticides, traffic exhaust, disinfectants), as well as by many different foods, drugs, ethanol and caffeine.

There has been considerable controversy over the existence of this phenomenon, punctuated by the many Gulf War veterans who have complained of similar symptoms and chemical intolerances since returning from the Gulf. Although there now is widespread recognition of MCS as a clinical phenomenon, there is still much uncertainty as to whether it is a definable syndrome, what its etiology is, and what biopsychosocial mechanisms might underlie it.

Relevant information about conditions like MCS is derived from three general sources, all of which supplement one another and each of which has its own limitations. Epidemiological studies often cannot differentiate which among several possible variables is (are) involved in the adverse effects observed. Once a toxic exposure has occurred, clinical research provides valuable information concerning diagnosis, therapy and rehabilitation. However, both epidemiology and clinical studies involve post hoc analyses, i.e. the human exposure has already occurred. A third

source of information in biomedical research — use of animal models—is the only approach which allows experimental manipulations and affords the precision of laboratory measurement. The major concern in using this approach is the validity of extrapolating results to human situations. Empirical tests of this validity have earned animal models an essential role in understanding human toxicities (Russell, 1991; MacPhail and Peele, 1992). Our present objective is to propose that use of a particular animal model will be useful in the search for the etiology of and mechanisms underlying MCS.

The validity of an animal model rests in part on its similarity in structure and function to the human situation. The closer the similarity, the greater is the probability that manipulations of one will provide information valid for extrapolation to the other. A final test of validity comes when predictions are shown to be accurate. To evaluate the model proposed below, it is important to summarize the observed clinical characteristics of MCS.

## 2. Multiple chemical sensitivity

Anecdotal descriptions of MCS have appeared in the medical literature for more than 40 years. In the past five years, occupational medicine physicians in universities have reported seeing increasing numbers of individuals with this affliction, and three federally-sponsored workshops on MCS have been held (Association of Occupational and Environmental Clinics, 1992; National Research Council, 1992; Mitchell and Price, 1994). Sponsoring agencies have included The National Research Council (NRC), the Agency for Toxic Substances and Disease Registry (ATSDR), the Environmental Protection Agency, and the National Institute of Environmental Health Sciences (NIEHS). Recommendations from these meetings have underscored the need for further research on the condition and the development of animal models.

MCS has been described as a two-step process that in some ways parallels allergic diseases (Ashford and Miller, 1991): (1) *Induction* (initiation,

sensitization or loss of tolerance) as a consequence of an initial chemical exposure (analogous to sensitization to bee venom), and (2) subsequent *triggering* of symptoms by a wide range of chemically-diverse substances (here the analogy to allergy breaks down, since antibodies are highly specific and spreading of sensitivities to chemically unrelated substances does not occur with allergy).

Among the substances MCS patients most frequently report as having induced their condition are pesticides, especially OPs and carbamates (Ashford and Miller, 1991; Miller and Mitzel, 1995). Interestingly, many Gulf War veterans also now report symptoms reminiscent of MCS. Exposures to OP and carbamate agents during the Gulf War included pesticides, the nerve agent pretreatment drug pyridostigmine bromide and, possibly, low levels of nerve agents. Although chemicals in this class can inhibit cholinesterase, rarely are cholinesterase levels measured in sporadic cases, and frequently acute symptoms associated with cholinesterase inhibition are absent among individuals who report developing MCS as a consequence of OP exposure. While OP toxicity has been considered largely reversible if it is not fatal, the toxicology literature contains numerous examples of individuals exposed to these agents who showed persistent neuropsychological deficits (Rowntree et al., 1950; Gershon and Shaw, 1961; Tabershaw and Cooper, 1966; Savage et al., 1988; Rosenstock et al., 1990). Some authors have proposed that OPs may damage cholinergic receptors or in other ways induce injury independent of their ability to inhibit cholinesterase (Gupta and Abou-Donia, 1994; Huff et al., 1994).

With respect to MCS, Rosenthal and Cameron (1991) described a retired attorney with depression whose home was sprayed with an OP pesticide. Subsequently, the attorney's depression became markedly more severe, and he developed multi-system symptoms and new intolerances to odors. These authors hypothesized that cholinergic sensitivity might underlie both environmental sensitivities and depressive tendencies. Cone and Sult (1992) described a group of casino workers exposed to a mixture of carbamate and

pyrethrin insecticides who subsequently developed chronic, multi-system symptoms, cognitive difficulties and sensitivity to the odor of pesticides, perfumes, gasoline, newsprint, and cleaning agents. Soon after their exposure, a number of these individuals, who were dealers, experienced difficulty counting cards. Later they reported feeling ill around tobacco smoke, an exposure they previously had tolerated while at work.

More recently, Miller and Mitzel (1995) surveyed 112 MCS patients, 37 of whom attributed their illness to exposure to an OP or carbamate pesticide and the other 75 to remodelling of a building. Remodelling commonly involves exposures to low levels of mixed solvents emanating from fresh paint, carpeting, glues, etc. Following their initial exposure, both groups reported similar symptoms and similar intolerances to chemicals, foods, ethanol, and caffeine. However, overall, the pesticide-exposed group reported significantly greater symptom severity. The authors interpreted these findings as suggesting a possible common pathway for the development of MCS, despite the fact that the two groups initially experienced exposures that were chemically different. They hypothesized that the relatively greater neurotoxicity or potency of the cholinesterase inhibitors versus mixed low-level solvents might explain the greater symptom severity in the pesticide-exposed group.

Notably, MCS patients frequently report that other individuals simultaneously exposed to pesticides, e.g. family members, friends, or co-workers, did not develop MCS or even experience transient illness. These observations suggest that a subset of the population may be more vulnerable to developing MCS. Some (Black et al., 1990; Simon et al., 1990), but not all (Fiedler et al., 1992) researchers have reported a greater rate of depression and somatization disorder that predated the "initiating" chemical exposure among persons with MCS compared to controls.

A vigorous medical debate has ensued, revolving around whether MCS is: (1) a physiological consequence of chemical exposure in biochemically susceptible individuals; (2) a psychological response to chemical exposure, e.g. a conditioned behavioral manifestation or post-

traumatic stress disorder; or (3) simply the patients' misattribution of some other illness, organically-based or not, to chemical exposures. Conceivably, depression, a prevalent symptom among MCS patients, could be, (1) a physiological or psychological risk factor for the development of MCS; (2) the consequence of patients' having to cope with a perplexing and disabling medical illness; or (3) one of many symptoms of MCS triggered by chemical exposure.

The FSL (Flinders Sensitive Line) rat was developed by selective breeding for increased sensitivity to an OP, so it shares some etiological similarity to patients with MCS who were exposed to pesticides. MCS patients, depressed patients and FSL rats exhibit many of the same intolerances for drugs and many of the same symptoms, as will be described below. The similarities and the extent to which the FSL rat can be considered a model for MCS will be discussed in a final section.

### 3. An animal model

The model is one with which we have had extensive experience, particularly in research on depressive syndromes (Overstreet and Janowsky, 1991; Overstreet, 1993). Analogies between depressed states and MCS, as well as substance intolerances in FSL rats, first brought our attention to the potential value of this model for experimental studies of MCS.

#### 3.1. Establishment of the model

The model arose from a selective breeding program designed to produce two lines of rats, one with high and one with low sensitivity to the anticholinesterase agent, diisopropylfluorophosphate (DFP) (Russell et al., 1982; Overstreet et al., 1979). The selective breeding program, which was initiated at Flinders University in Adelaide, Australia, utilized decreases in core body temperature, drinking and body weight to measure sensitivity to DFP. A rank-order system was used to give equal weighting to each of the three variables. Rats which had the lowest average ranks were intermated to establish and maintain

the line of more sensitive rats (Flinders Sensitive Line—FSL), while rats which had the highest average ranks were intermated to establish and maintain the line of more resistant rats (Flinders Resistant Line—FRL). Subsequent studies showed that randomly bred Sprague-Dawley rats, from which the lines were originally derived, were not different from the FRL rats. On the other hand, FSL rats were significantly more sensitive to DFP than the other two groups (Russell et al., 1982; Overstreet et al., 1979).

Because early studies ruled out changes in acetylcholinesterase as a mechanism to account for the differential sensitivity of FSL and FRL rats to DFP (Overstreet et al., 1979; Russell and Overstreet, 1987), the effects of other muscarinic agonists on these rats were examined (Overstreet and Russell, 1982; Overstreet, 1986; Overstreet et al., 1986a,b). These studies showed that the FSL rats were more sensitive to pilocarpine, arecoline and oxotremorine than were the FRL rats; this supersensitivity was seen for a variety of responses, including hypothermia, reduced locomotor activity, and suppression of bar-pressing for water reward (Overstreet and Russell, 1982). Biochemical studies indicated that the FSL rats exhibited greater numbers of muscarinic receptor binding sites in the hippocampus and striatum than the FRL rats (Overstreet et al., 1984; Pepe et al., 1988).

As selective breeding progressed, animals were shipped to the University of North Carolina at Chapel Hill (UNC), where colonies were started. Here, young from the FSL and FRL rats were cross-fostered onto pathogen-free mothers in order to establish pathogen-free colonies. Over the last ten generations of FSL and FRL rats bred at the University of North Carolina (S46-S55), there has been no overlap in the two distributions, indicating that the lines are still quite separate for this phenotype.

#### 3.2. Differences in activity after stressors

FSL rats have been reported to have lower locomotor activity than the FRL rats under a number of experimental conditions (Overstreet and Russell, 1982; Overstreet, 1986; Bushnell et al., 1995) but not all (Criswell et al., 1994; Rez-

vani et al., 1994). Those situations which failed to report a difference in activity between the lines involved automated recording systems over long (>1 h) durations in environments to which the animals had become habituated. In contrast, those studies reporting differences in activity employed short exposures (<5 min) to an open field apparatus. The reduced activity of the FSL rats, at least in males, is commonly seen under baseline conditions in these novel environments (Overstreet and Russell, 1982; Overstreet, 1986), a parallel to euthymic depressed humans (Wolff et al., 1985). In addition, however, locomotor activity is suppressed to an even greater degree when the FSL rats are exposed briefly (2 s) to a mild (1 mA) foot shock (Overstreet, 1986; Overstreet et al., 1989a). In particular, both male and female FSL rats exhibited a similar degree of locomotor suppression after foot shock, even though only the male FSL rats exhibited lower baseline activities (Overstreet, 1986). These data have led to the suggestion that the FSL rat is an animal model of genetic predisposition toward depression, in that dramatically decreased locomotor activity is seen only upon exposure to stressors (Anisman and Zacharko, 1982; Overstreet et al., 1988; Overstreet and Janowsky, 1991).

Results from several other behavioral paradigms are consistent with the view that depressive-like psychomotor retardation symptoms are more apparent in the FSL rats after exposure to stressors. For example, the FSL rats are impaired in active avoidance paradigms compared to the FRL rats (Overstreet and Measday, 1985; Overstreet et al., 1990a, 1992). The reduced ability of the FSL rats to acquire a shock-motivated task is completely consistent with the above results which show a much greater suppression of activity in the FSL rats after exposure to shock. An alternative interpretation, that there are differences in "anxiety" (immobility after shock or in novel environments) between the FSL and FRL rats (Criswell and Breese, 1989) is not supported by experimental results in the elevated plus maze (Schiller et al., 1991).

Another stress-oriented paradigm which has provided important information about behav-

ioral differences between FSL and FRL rats is the forced swim test. Upon initial exposure in a cylinder (18–20 cm diameter) of water (25°C), FSL rats are more immobile than the FRL rats (Overstreet, 1986; Overstreet et al., 1986a; Schiller et al., 1992; Pucilowski and Overstreet, 1993). This exaggerated immobility of the FSL rats is counteracted by chronic but not acute treatment with antidepressants (Schiller et al., 1992; Overstreet, 1993; Pucilowski and Overstreet, 1993). These findings provide further support for the contention that the FSL rat is a useful animal model of depression.

It might be argued that the exaggerated immobility exhibited by the FSL rats in the forced swim test could indicate that they fatigue more easily, or suffer from a loss of energy, as do human depressives. However, psychomotor retardation as such is a more likely explanation for exaggerated immobility because, as indicated above, differences in immobility between the two lines can be seen very quickly in the forced swim test. Similarly, because exposures to the open field test are also brief, fatigue is probably not a factor. Recently, the FSL and FRL rats have been compared in a treadmill task. The FSL rats became exhausted after a shorter period of exercise on the treadmill than the FRL rats (Bailey et al., 1994), a possible indicator of fatigability. Thus, FSL rats appear to fatigue more readily, which may be a parallel to the well known fatigue found in depressed humans.

### 3.3. Differences in reward-related behaviors

There are also differences in reward-related behaviors between the FSL and FRL rats which are consistent with the proposal that the FSL rats are a model of depression. In an operant bar-pressing task for water reward, the FSL rats learned the task as readily as did the FRL rats, but stabilized at a much lower response rate in the 15-min session even though both groups were deprived of fluid for 23.5 h per day (Overstreet and Russell, 1982). More recently, Bushnell et al. (1995) reported that the FSL rats were much slower to respond in a food-motivated match-to-sample learning task and they became satiated more readily. The FSL rats had to be maintained

at a lower percentage of their free-feeding body weight and have smaller food pellets (37 vs. 45 mg) in order to keep their motivation sufficiently high to complete the session (Bushnell et al., 1995). In some cases, there are not any baseline differences in the FSL and FRL rats in reward-related tasks. For example, the two lines exhibit similar preferences for saccharin solution over water in a two-bottle choice paradigm, but FSL rats exhibit greater decreases in saccharin preference following exposure to stressors (Pucilowski et al., 1993).

#### 3.4. Differences in cognitive behavior

Actively depressed individuals commonly complain about their inability to concentrate and cognitive impairment is frequently associated with the depressed mood. The data on cognitive (learning) impairments in the FSL rats are mixed. Initially, it was found that the FSL rats exhibit much better memories in the passive avoidance paradigm, where a good performance is reflected by the rat remaining stationary (Overstreet, 1986). In contrast, the FSL rat has difficulty acquiring an active avoidance response (Overstreet et al., 1990a, 1992). Since both of these outcomes could be accounted for by the tendency of the FSL rats to inhibit activity after exposure to shock (see above), an appetitive paradigm was used. In this food-motivated matching-to-sample task, the FSL rats exhibited slower responding, but their choice accuracy was not different from that of the FRL rats (Bushnell et al., 1995). Therefore, it must be concluded that the FSL rat does not exhibit the same degree of cognitive impairment as seen in actively depressed humans.

#### 3.5. Differences in sleep

Insomnia, or an inability to sleep, is a symptom commonly reported in depression. There are many forms of insomnia, but the most common complaint of depressives appears to be one of early morning awakening and/or fragmented sleep without being able to return to sleep (Whybrow et al., 1984). It is rather difficult to model these more subjective aspects of sleep. However, there are more complex changes in the sleep-wake cycle of depressed humans which can

be determined by polysomnography studies (Gillin et al., 1979, 1981; Benca et al., 1992). Such studies indicate that sleep in depression can be differentiated from non-depressive sleep (Gillin et al., 1979), and that depression is associated with a reduction in the deeper stages (3 and 4) of sleep, a reduction in the latency to the first episode of rapid eye movement (REM) sleep, and an increase in the amount or density of REM sleep (Kupfer, 1976; Gillin et al., 1979, 1981; Benca et al., 1992). Human depressives are also more sensitive to the effects of cholinergic agonists on REM sleep latency (Janowsky et al., 1994).

Several days of 24 h sleep recordings in FSL and FRL rats have been carried out under baseline undisturbed conditions. These studies indicated that FSL rats exhibited significantly more REM sleep than FRL rats, but there were no differences in the amount of slow wave sleep (Shiromani et al., 1988). In addition, there was a significantly shorter interval between REM episodes in FSL rats. Thus, neither FSL rats nor depressed humans exhibit an overall reduction in sleep time, but both exhibit increases in REM sleep and reductions in REM sleep onset. The selected differences in REM sleep between the FSL and FRL rats have been replicated using a sleep deprivation paradigm (Shiromani et al., 1991) and an automated scoring system (Benca et al., 1993). Interestingly, persistent REM sleep abnormalities have been noted in depressed humans following clinical recovery from depression (Benca et al., 1992), a finding which parallels the "trait" aspects of the FSL rat's "sleep disorder".

#### 3.6. Trait/state considerations

It has been recognized for some time that many biological changes seen in depressed individuals are present only while they are actively depressed (state changes), while others can be observed even after the individual has recovered (trait characteristics; Janowsky et al., 1994). Therefore, if the FSL rat is a true genetic animal model of depression, then it should not be "depressed" all the time. Most individuals suffering from depressive disorders have long periods of essentially normal behavior between episodes. Study of the FSL animals under various manipu-

lations may help to understand how genetic and environmental variables interact to produce depressive-like phenomena. Thus, the REM sleep changes observed in FSL rats may reflect an underlying trait predisposing to depression (Benca et al., 1992; Janowsky et al., 1994). Reduced activity can be seen in FSL rats under baseline conditions, just as has been observed in euthymic depressed patients (Wolff et al., 1985); however, the reduced activity in both FSL rats and depressed humans is much more prominent after exposure to stressors. Finally, studies suggest that superimposing the chronic mild stress paradigm upon this genetic model has resulted in data indicating that FSL rats appear to be more "anhedonic" than FRL rats since the former exhibit a significantly greater decrease in saccharin preference (Pucilowski et al., 1993). An unanswered question is whether the anhedonia lasts for a longer period of time in the FSL rats.

In sum, the FSL rats and depressed humans exhibit a large number of behavioral and physiological similarities (see Overstreet, 1993, for a more detailed description).

### 3.7. Drug interactions

Clinical observations suggest that MCS may be initiated by acute or chronic exposure to diverse chemical agents. As described earlier, the FSL rats were selectively bred to have increased responses to the anticholinesterase agent, DFP, so it should not be surprising that they also exhibit increased sensitivity to muscarinic agonists (Overstreet and Russell, 1982; Overstreet, 1986; Schiller et al., 1988; Daws et al., 1991; Overstreet et al., 1992a,b). Notably, there have also been several reports of increased sensitivity to anticholinesterases in human depressives (Janowsky and Risch, 1987; Sitaram et al., 1987; Nurnberger et al., 1989; Gann et al., 1992; O'Keane et al., 1992) and MCS patients (Rosenthal and Cameron, 1991; Cone and Sult, 1992; Miller and Mitzel, 1995). Human depressives are also more sensitive to directly acting muscarinic agonists (Gillin et al., 1991; Gann et al., 1992); according to a recent report, so are children of depressives (Schreiber et al., 1992). At present there are no published data for MCS patients

regarding sensitivity to direct cholinergic agonists in particular, but such agents are among those which many MCS patients say they cannot tolerate.

Knowledge about possible serotonergic alterations in the FSL rats has increased along with the availability of more selective agonists. In early studies, only relatively nonselective drugs were available, and the FSL rats were found to exhibit a greater degree of hypothermia after both *m*-chlorophenylpiperazine (mCPP), a 5-HT<sub>1B/C</sub> agonist, and cyproheptadine, a nonselective 5-HT (5-hydroxytryptamine) antagonist (Wallis et al., 1988). It was argued that these results favored the hypothesis of a 5-HT<sub>1</sub> supersensitivity in the FSL rats because 5-HT<sub>1</sub> agonists were reported to produce hypothermia while 5-HT<sub>2</sub> agonists induced hyperthermia (Gudelsky et al., 1986). Subsequent studies exploring the hypothermic effects of buspirone and 8-OH-DPAT, selective 5-HT<sub>1A</sub> agonists, confirmed the 5-HT<sub>1</sub> supersensitivity in the FSL rats (Overstreet et al., 1992). However, using an operant responding paradigm, Schiller (1991) found that the FSL rats were more sensitive to the behavioral suppressant effects of both quipazine, a 5-HT<sub>2/1C</sub> agonist, and mCPP, a 5-HT<sub>1B/C</sub> agonist. In addition, he reported preliminary evidence for increases in cortical 5-HT receptors of both subtypes in the FSL rats (Schiller, 1991). Thus, there is considerable evidence for serotonergic supersensitivity in the FSL rats, especially of the 5-HT<sub>1</sub> subtype. This outcome is consistent with much of the evidence suggesting supersensitive serotonergic mechanisms in depressives (Arora and Meltzer, 1989; Arango et al., 1990; Mikuni et al., 1991), but is not consistent with neuroendocrine studies reporting blunted responses to serotonergic agonists, which suggests serotonergic hyposensitivity (Meltzer and Lowy, 1987; Lesch et al., 1990). As yet, there are no data on the effects of selective serotonergic agents in MCS patients, so the similarity between the FSL rats and MCS patients for this parameter cannot be evaluated at present.

If the FSL rat model of depression mimicked the changes in noradrenergic function seen to occur in depressive disorders, then FSL rats

should be supersensitive to beta-noradrenergic agonists and subsensitive to alpha-noradrenergic agonists. To date, there has been no support for this hypothesis in the FSL/FRL rats. The reductions in body temperature and in locomotor activity induced by the beta-noradrenergic agonist salbutamol were similar in the FSL and FRL rats (Overstreet et al., 1989a). Similarly, the reductions in body temperature and in operant responding for water reward induced by the alpha-noradrenergic agonist clonidine were also similar in the FSL and FRL rats (Overstreet, 1989). Thus, even though the studies to date are quite limited, there do not appear to be any marked differences in behaviorally represented noradrenergic function between FSL and FRL rats.

Interactions with the dopaminergic system would be expected because of the dopamine deficiency observed in human depressives (Wise, 1979; Willner, 1983). The prediction is that depressed humans and FSL rats should be supersensitive to the effects of dopamine agonists. The

data collected so far on dopaminergic mechanisms is partially consistent with this hypothesis. The FSL rats are supersensitive to the hypothermic (Crocker and Overstreet, 1991) and aggression-promoting (Pucilowski et al., 1991) effects of apomorphine, a mixed D1/D2 agonist, and quinpirole, a selective D2 agonist. On the other hand, the FSL rats are subsensitive to the stereotypy-inducing effects of apomorphine and quinpirole at similar doses where supersensitivity to the hypothermic effects were seen (Crocker and Overstreet, 1991). In addition, no evidence for differences in dopamine D2 receptors between FSL and FRL rats could be detected in a series of studies (Crocker and Overstreet, 1991). Consequently, it was argued that the opposite changes in sensitivity in the various functions could be related to the way the cholinergic and dopaminergic systems interact to modulate those functions. Both cholinergic and dopaminergic stimulation promote hypothermic and aggressive responses (Cox et al., 1980; Pucilowski, 1987; Ray et al., 1989), but cholinergic stimulation has op-

Table 1  
Multiple chemical sensitivity in FSL rats

Compound	Mechanism of action	Responses
Conditions under which FSL rats are more sensitive than FRL rats to drugs		
DFP	Anticholinesterase	Temperature/drinking
Physostigmine	Anticholinesterase	Temperature/activity
Oxotremorine	Muscarinic agonist	Temperature/activity
Pilocarpine	Muscarinic agonist	Temperature/activity
Arecoline	Muscarinic agonist	Temperature/activity
Nicotine	Nicotinic agonist	Temperature/activity
Apomorphine	Dopamine D1/2 agonist	Temperature
Quinpirole	Dopamine D2 agonist	Temperature
Raclopride	Dopamine D2 antagonist	Catalepsy
mCPP	5-HT-1B agonist	Temperature/activity
8-OH-DPAT	5-HT-1A agonist	Temperature
Buspirone	5-HT-1A agonist	Temperature
Diazepam	Benzodiazepine agonist	Temperature/activity
Ethanol	Multiple (GABA, 5-HT)	Temperature
Conditions under which FSL rats are less sensitive than FRL rats to drugs		
Scopolamine	Muscarinic antagonist	Activity
Apomorphine	Dopamine D1/2 agonist	Stereotypy
Quinpirole	Dopamine D2 agonist	Stereotypy
MK-801	NMDA antagonist	Temperature

**Table 2**  
Effects of blocking drugs on ethanol-induced hypothermia in Flinders Sensitive and Resistant rats

Compound	Dose	Decrease in temperature (°C, mean ± S.E.M.)		
		FSL rats	FRL rats	Difference
<b>Experiment 1</b>				
Ethanol (E) only	3 g/kg	2.8 ± 0.5	1.6 ± 0.5	1.2
E + scopolamine	1 mg/kg	2.6 ± 0.5	1.6 ± 0.4	1.0
E + bicuculline	2 mg/kg	2.7 ± 0.6	1.6 ± 0.5	1.1
<b>Experiment 2</b>				
Ethanol only	3 g/kg	2.3 ± 0.5	1.2 ± 0.4	1.1
E + verapamil	10 mg/kg	3.0 ± 0.6*	1.8 ± 0.5*	1.2
E + nicardipine	10 mg/kg	2.2 ± 0.5	0.9 ± 0.6	1.3
E + haloperidol	0.5 mg/kg	2.1 ± 0.5	1.0 ± 0.5	1.1
E + naltrexone	2 mg/kg	2.3 ± 0.7	1.0 ± 0.5	1.3
E + pindolol	1 mg/kg	1.9 ± 0.8	1.0 ± 0.5	0.9
E + mecamlamine	5 mg/kg	5.6 ± 1.2*	2.8 ± 0.8*	2.8**
E + hexamethonium	5 mg/kg	3.3 ± 1.1*	1.9 ± 0.9*	1.2
<b>Experiment 3</b>				
Ethanol only	3 g/kg	2.9 ± 0.3	1.7 ± 0.3	1.2
E + idazoxan	3 mg/kg	5.2 ± 0.3*	4.0 ± 0.3*	1.2
E + nicotine	0.4 mg/kg	4.0 ± 0.4*	2.5 ± 0.3*	1.5**

\*Significantly greater than ethanol only; potentiated hypothermia.

\*\*Greater difference in temperature than with ethanol only.

posite effects to dopaminergic stimulation in the modulation of activity and stereotypy (Fibiger et al., 1970; Klemm, 1989).

In addition to the above drugs which interact selectively with specific neurotransmitter receptors, the FSL and FRL rats are differentially sensitive to the effects of several other pharmacological agents, as summarized in Table 1. However, as with the case of dopamine agonists, the differential effects are observed only for some actions of the drugs, not for all. For example, ethanol induces a greater hypothermia in the FSL rats, but not a greater intoxication (Overstreet et al., 1990b). Similarly, diazepam produces greater behavioral suppressant effects in the FSL rats (Pepe et al., 1988), but the anxiolytic effects of diazepam in the two lines are comparable (Schiller et al., 1991).

We pretreated FSL and FRL rats with antagonists selective for specific neurotransmitters in an attempt to determine if the differences in

ethanol-induced hypothermia in the two lines were related to particular neurotransmitter differences. The results of this study (previously unpublished) are shown in Table 2. Although some compounds altered the hypothermic effects of ethanol, there were parallel changes in the two lines and no agent reduced the 1°C difference between the two lines. Surprisingly, the nicotine antagonist, mecamlamine, dramatically potentiated the hypothermic effects of ethanol more in the FSL than the FRL rats. These findings provide extensive replication of the previously reported differences in ethanol-induced hypothermia between the FSL and FRL rats (Overstreet et al., 1990b), but have failed to elucidate the mechanism underlying this differential effect.

In summary, it is quite clear that the FSL rat is more sensitive to a variety of chemical agents in addition to the anticholinesterase for which they were selectively bred. In this regard, the FSL rat is, in part, analogous to MCS patients who

become more sensitive to a range of agents following exposure to OP anticholinesterases. The extent of the similarity between the FSL rats and MCS patients, on one hand, and human depressives and MCS patients, on the other, will be evaluated in the next section.

#### 4. Mechanisms of action

As Table 3 summarizes, the behavioral features of individuals with MCS and those of depressed patients and FSL rats are strikingly similar in regard to weight, appetite, activity and stressability, hedonia, and sleep. A closer look at Table 3 suggests several studies that might be carried out in MCS patients to test further the extent of the associations among the three groups. For example, polysomnographic recordings of sleep in asymptomatic MCS patients would be particularly informative, especially since there is evidence that the REM sleep changes seen in depressed patients may be a trait marker of this disorder (Benca et al., 1992; Janowsky et al., 1994). So far, information about sleep in MCS patients is of a more subjective nature. However Bell (1995) has recently reported a decrease in slow wave sleep in individuals with odor intolerances, a finding found in human depressives but not FSL rats. Since REM sleep alterations can also be related to altered cholinergic mechanisms in general (Shiromani et al., 1987; Janowsky et al., 1994), a finding of REM sleep changes in

MCS patients would suggest that altered cholinergic mechanisms might underlie abnormal sensitivity to chemicals. Such a finding would also be consistent with a cholinergic hypothesis as one possible explanation for the similarity between the MCS patients and depressives.

Another similarity between MCS and depression is that there are many more females than males expressing the symptoms (Table 3). Twice as many females than males report depressive symptoms, but the incidence of bipolar illness is equal in the genders (Goodwin and Jamison, 1990). The ratio of female to male MCS patients is even higher, reaching 4/1 in some studies (Miller and Mitzel, 1995). The information on cholinergic sensitivity in the female and male FSL rats cannot easily be related to the human data, because sensitivity is a continuous variable and neither the female nor the male populations overlap with their FRL counterparts (Overstreet, 1993). However, adult female FSL rats are more sensitive to cholinergic agonists than their male counterparts (Netherton and Overstreet, 1983). The greater sensitivity of adult females to cholinergic agonists might therefore contribute to the greater incidence of depression (Overstreet et al., 1988) and MCS in this gender.

Given the behavioral similarities between MCS and depressed patients (Table 3), one could predict that depressed patients might be hypersensitive to various drugs. Unfortunately, as described in Table 4, there is not much information

Table 3  
Comparison of characteristics and behavioral features of MCS patients, FSL rats and depressed patients

Measure	MCS patients	FSL rats	Depressed patients
Weight	Up or down	Down	Up or down
Appetite	Up or down	Down	Up or down
Blood pressure	Up or down	ND	Up or down
Food craving	++	+	+
Sleep disturbances	+++	++	+++
Loss of drive	+++	+++	+++
Reduced activity	+++	+++	+++
Cognitive disturbance	+++	+/-	+++
Gender ratios (F/M)	4/1	F > M	2/1

ND, not determined

Table 4  
Comparison of drug sensitivity in MCS patients, FSL rats and depressed patients

Compound	MCS patients	FSL rats	Depressed patients
Anticholinesterases	+++	+++	+++
Solvents, etc.	+++	ND	ND
Ethanol	+++	++	+
Nicotine	+++	++	+
Xanthines	+++	ND	ND
Foods	+++	ND	ND

ND, not determined.

concerning the sensitivity of depressed individuals to the range of drugs reported to cause problems in MCS patients, other than their supersensitivity to anticholinesterases and cholinergic agonists (Janowsky et al., 1994). There is somewhat more evidence for a general increase in sensitivity to drugs in the FSL rats (Tables 1 and 4). It is particularly noteworthy that the FSL rats are more sensitive to both alcohol (Overstreet et al., 1990b) and nicotine (Schiller and Overstreet, 1993). The information on the effects of alcohol and nicotine in depressed patients is more complex, as implied by the question mark in Table 4. While we are not aware of any studies specifically stating that depressed patients report intolerances for alcohol and/or nicotine, there are much data related to the interaction of depression with primary alcoholism on one hand (e.g. Schuckit, 1986; Kendler et al., 1993; Maier et al., 1994) and to the interaction of smoking with depression on the other (Breslau et al., 1991; Glassman, 1993).

It should be stressed that FSL rats are also less sensitive to certain drugs (Table 1) and that depressed patients exhibit blunted hormonal responses to a number of drugs affecting serotonergic and noradrenergic mechanisms (Meltzer and Lowy, 1987). Therefore, there is a need to collect more data from depressed individuals and FSL rats on their sensitivities to a broader range of chemicals. If the cholinergic system is a link between the three conditions, then it would be predicted that both FSL rats and depressed individuals would be more sensitive to such drugs. Also needed is more data on depressed individuals and FSL rats with respect to the triggering

of symptoms by chemical or food exposures (Table 4).

Although we have emphasized the possibility of a cholinergic link between MCS patients, depressed patients, and FSL rats, other neurotransmitter systems may be involved. Serotonin (5-hydroxytryptamine; 5-HT) has been implicated in depression (Meltzer and Lowy, 1987) and recent experiments on the Flinders rats suggest that serotonergic mechanisms may play an important role in some of their altered behaviors (Overstreet et al., 1994). We can only speculate about the role of serotonergic mechanisms in MCS patients, as there are no data. However, given the wealth of information on serotonergic mechanisms in depressed patients, it should be possible to design and conduct appropriate experiments to address this possibility in MCS patients.

A somewhat more complex neurotransmitter model proposes that the various neurochemical systems interact with one another and that abnormal behavioral states may arise from an alteration in one system which creates an imbalance in interactions with others. The original concept proposed by Janowsky et al. (1972) suggested that depression and mania were the consequence of imbalances between the noradrenergic and cholinergic systems, with depression being associated with relative cholinergic overactivity and mania being associated with relative noradrenergic overactivity (see also Fibiger et al., 1970). This model could account for some of the effects observed in the FSL rats following administration of noncholinergic drugs. For example, FSL

rats are more sensitive to the hypothermic effects of dopamine agonists, but less sensitive to their stereotypy-inducing effects (Table 1). Since dopaminergic and cholinergic systems work in parallel to regulate temperature but in opposition to regulate activity and stereotypy, an overactive cholinergic system could account for the findings with the dopamine agonists (Overstreet, 1993).

Another type of mechanism which could underlie all three conditions is a change in second messenger rather than neurotransmitter functions. Several investigators have proposed that changes in G proteins, cyclic AMP or other second messenger systems may be involved in depression (Wachtel, 1989; Lesch and Manji, 1992; Avissar and Schreiber, 1993). It has been suggested that the functional muscarinic responses in the FSL and FRL rats are too divergent to be explained by the relatively small differences noted in muscarinic receptors (Overstreet, 1993). If correct, such a hypothesis may more easily account for the pervasiveness of the chemical sensitivity described in MCS patients, which involves many classes of chemical compounds besides those having direct effects on neurotransmitter systems. Differences in second messengers could be hereditary or induced by exposure to chemical agents. Further study of FSL rats, MCS patients, and depressed patients using diverse approaches is needed to obtain a clear picture of the mechanisms that may underlie MCS.

## 5. A proposal for future studies

In conclusion, we propose that the characteristics of the animal model we have described are sufficiently analogous to MCS to warrant its use in testing hypotheses about the etiology and mechanisms of action involved in the syndrome. An example of the type of experimental protocols suggested by this review is the study of FSL and FRL rats after exposure to volatile solvents and other chemicals to which MCS patients report intolerance. If FSL rats do exhibit increased sensitivity to a wide variety of chemical agents, then treatment approaches could be attempted

using antidepressant drugs, for example. It should be emphasized that proposing antidepressant treatment does not presume that depression is the cause of MCS; quite the reverse might be true. For example, exposure to OPs might augment cholinergic sensitivity, leading to both MCS and depression. The possibility that increased cholinergic sensitivity might underlie both MCS and depression suggests further experiments in these patient groups. Is there a subset of depressed patients who report intolerance to varied substances? Do these same patients exhibit a greater sensitivity to cholinergic agents? Would this subset of depressed patients benefit from avoidance of certain drugs and environmental exposures? Do MCS patients have altered cholinergic responsivity?

These experimental approaches can provide information which is not obtainable by other methods and which, therefore, could add significantly to our knowledge of a disabling syndrome, one which is strikingly similar to the illness reported among Gulf War veterans (Miller, 1994b; Miller and Mitzel, 1995). As Dr. Louis W. Sullivan, then U.S. Health and Human Services Director, stated at an Experimental Biology meeting in 1990:

humanely conducted animal research is critically important in our search for cause and treatments for AIDS, cancer, Alzheimer's disease, schizophrenia and other diseases, just as it was critically important for virtually every major biomedical discovery in the past.

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