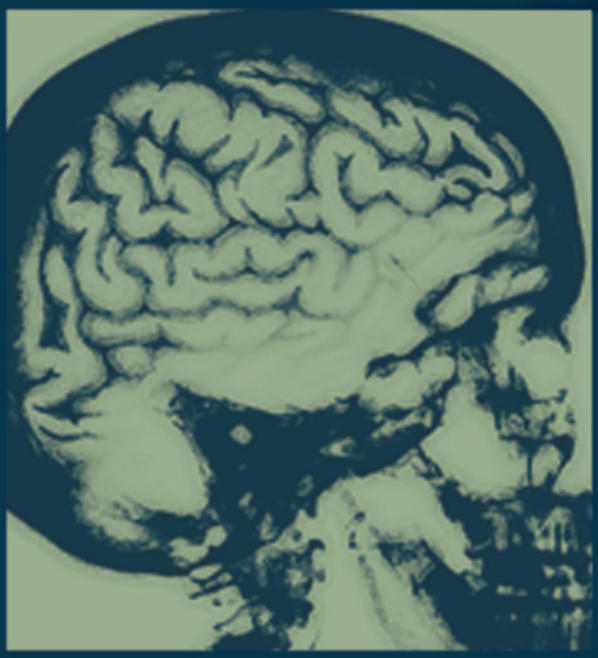
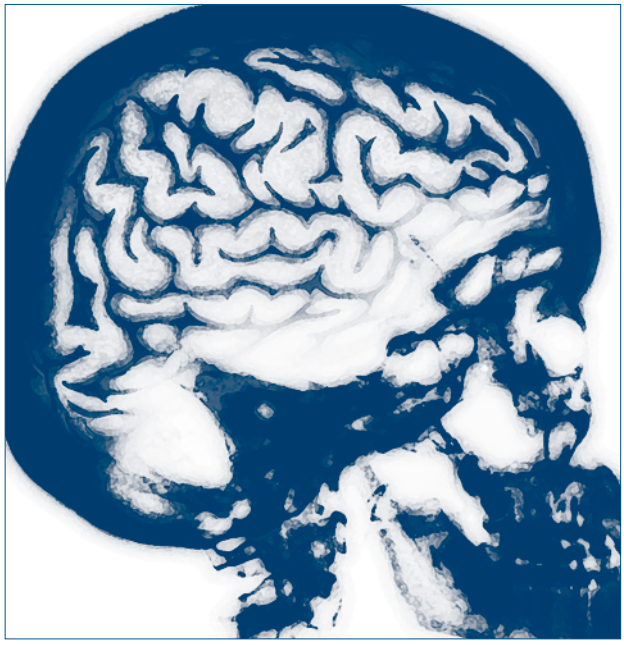


Neuroimmune Mechanisms and Chronic Fatigue Syndrome:

Will Understanding Central Mechanisms Enhance the Search for the Causes, Consequences, and Treatment of CFS?

June 12–13, 2003

A Report of the Scientific Workshop Co-Sponsored by
the NIH Office of Research on Women's Health and
the Trans-NIH Working Group for Research on Chronic Fatigue Syndrome



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Contents

Speakers

Gail Kurr Adler, M.D., Ph.D.
Assistant Professor
Department of Medicine
Brigham and Women's Hospital
221 Longwood Avenue
Boston, MA 02115
Phone: 617-732-5661
Fax: 617-732-5764
E-mail: gadler@partners.org

Lesley M. Arnold, M.D.
Associate Professor
University of Cincinnati
College of Medicine
231 Albert Sabin Way
Cincinnati, OH 45267-0559
Phone: 513-558-4622
Fax: 513-558-4280
E-mail: lesley.arnold@uc.edu

Dedra Buchwald, M.D.
Professor of Medicine
University of Washington
Harborview Medical Center
325 Ninth Avenue
Box 359780
Seattle, WA 98104
Phone: 206-341-4439
Fax: 206-731-8247
E-mail: dedra@u.washington.edu

Daniel Clauw, M.D.
Professor of Medicine
University of Michigan
24 Frank Lloyd Wright Drive
Box 385
Ann Arbor, MI 48106
Phone: 734-998-6961
Fax: 734-998-6900
E-mail: dclauw@med.umich.edu

Leslie J. Crofford, M.D.
Associate Professor of Internal Medicine
University of Michigan
Room 5520 MSRB-1
1150 West Medical Center Drive
Ann Arbor, MI 48109-0680
Phone: 734-936-3257
Fax: 734-763-2025
E-mail: crofford@umich.edu

Firdaus S. Dhabhar, Ph.D.
Associate Professor
Ohio State University
4179 Postle Hall
305 West Twelfth Avenue
Columbus, OH 43210
Phone: 614-688-8562
Fax: 614-247-6945
E-mail: dhabhar.1@osu.edu

David Goldstein, M.D., Ph.D.
Chief, Clinical Neurocardiology Section
National Institute of Neurological Disorders
and Stroke
National Institutes of Health
10 Center Drive, MSC-1620
Building 10, Room 6N252
Bethesda, MD 20892-1620
Phone: 301-496-2103
Fax: 301-402-0180
E-mail: goldsteind@ninds.nih.gov

Eleanor Hanna, Ph.D.
Associate Director
Special Projects and Centers
Office of Research on Women's Health
National Institutes of Health
6120 Executive Boulevard, Room 150A
Bethesda, MD 20892-7116
Phone: 301-435-1573
Fax: 301-402-0005
E-mail: hannae@od.nih.gov

Margaret M. Heitkemper, RN, Ph.D.
Professor
University of Washington
1959 NE Pacific
Seattle, WA 98195
Phone: 206-543-1091
Fax: 206-543-4771
E-mail: heit@u.washington.edu

Nancy G. Klimas, M.D.
Professor of Medicine
University of Miami VA Medical Center
1201 NW 16th Street (111-I)
Miami, FL 33125
Phone: 305-575-3267
Fax: 305-575-3139
E-mail: nancy.klimas@med.va.gov

James Krueger, Ph.D.
Professor
Washington State University
College of Veterinary Medicine
264 Wagner
Pullman, WA 99164-6520
Phone: 509-335-8212
Fax: 509-335-4650
E-mail: Krueger@vetmed.wsu.edu

Juan F. López, M.D.
Associate Professor
Department of Psychiatry
Assistant Research Scientist
University of Michigan
Mental Health Research Institute
205 Zina Pitcher Place
Ann Arbor, MI 48109-0720
Phone: 734-615-3144
Fax: 734-647-4130
E-mail: jflopez@umich.edu

Harvey Moldofsky, M.D., FRCP(c)
Professor
Sleep Disorders Clinic
Centre for Sleep and Chronobiology
340 College Street
Suite 580
Toronto, Ontario M5T 3A9
Phone: 416-603-9531
Fax: 416-603-2388
E-mail: h.moldofsky@utoronto.ca

Mark R. Opp, Ph.D.
Associate Professor
Anesthesiology and Molecular and
Integrative Physiology
University of Michigan
1150 West Medical Center Drive
7433 MS Building 1
Ann Arbor, MI 48109-0615
Phone: 734-615-8768
Fax: 734-764-9332
E-mail: mopp@umich.edu

Dimitris A. Papanicolaou, M.D.
Assistant Professor of Medicine
Department of Medicine/Endocrinology
Emory University
Room 1307, West Wing WMRB
1639 Pierce Drive
Atlanta, GA 30322
Phone: 404-727-1395
Fax: 404-727-1300
E-mail: dpapani@emory.edu

Denise Park, Ph.D.
University of Illinois at Urbana–Champaign
Beckman Institute
405 North Mathews Avenue
Urbana, IL 61801
Phone: 217-244-6348
Fax: 217-244-9381
E-mail: denisep@uicu.edu

Vivian W. Pinn, M.D.
Director
Office of Research on Women's Health
National Institutes of Health
9000 Rockville Pike
Building 1, Room 201
Bethesda, MD 20892
Phone: 301-402-1770
Fax: 301-402-1798
E-mail: pinnv@od.nih.gov

Gary S. Richardson, M.D.
Senior Research Scientist
Henry Ford Hospital
Clara Ford Pavilion–3
2799 West Grand Boulevard
Detroit, MI 48202
Phone: 313-916-5182
Fax: 313-916-5167
E-mail: gsr@attglobal.net

Lee Simon, M.D.
Director
Division of Anti-Inflammatory, Analgesic, and Ophthalmic Drug Products
Food and Drug Administration
Office of Drug Evaluation V
Center for Drug Evaluation and Research
9201 Corporate Boulevard, N-314, HFD-550
Rockville, MD 20850
Phone: 301-827-2017
Fax: 301-827-2531
E-mail: simonl@cder.fda.gov

Esther M. Sternberg, M.D.
Director, Integrative Neural Immune Program
Chief, Section on Neuroendocrine Immunology & Behavior
National Institute of Mental Health
National Institutes of Health
36 Convent Drive
Room 1A 23, MSC-4020
Bethesda, MD 20892-4020
Phone: 301-496-9255
Fax: 301-496-6095
E-mail: ems@codon.nih.gov

Linda A. Toth, D.V.M., Ph.D.
Professor of Pharmacology
Southern Illinois University
School of Medicine
801 North Rutledge Street
Box 19611
Springfield, IL 62794-9611
Phone: 217-545-3053
Fax: 217-545-6263
E-mail: ltoth@siumed.edu

Suzanne D. Vernon, Ph.D.
Research Microbiologist
Centers for Disease Control and Prevention
1600 Clifton Road, MSA 15
Atlanta, GA 30333
Phone: 719-539-7828
Fax: 719-539-2058
E-mail: svernon@cdc.gov

Peter D. White, M.D., FRCP, FRCPsych
Barts and The London Queen Mary School of Medicine and Dentistry
University of London
Department of Psychological Medicine
St. Bartholomew's Hospital
London EC1A 7BE
United Kingdom
Phone: 44 (0) 207 601 8108
Fax: 44 (0) 207 601 7969
E-mail: P.D.White@qmul.ac.uk

David A. Williams, Ph.D.
Associate Professor of Medicine
University of Michigan
24 Frank Lloyd Wright Drive
Box 385
Ann Arbor, MI 48106
Phone: 734-998-6865
Fax: 734-998-6900
E-mail: daveawms@umich.edu

Jon-Kar Zubieta, M.D., Ph.D.
Associate Professor
University of Michigan
MHRI
205 Zina Pitcher Place
Ann Arbor, MI 48109-0720
Phone: 734-763-6843
Fax: 734-647-4130
E-mail: zubieta@umich.edu

Welcome

Vivian W. Pinn, M.D.
Associate Director for Research on Women's Health, National Institutes of Health

I am delighted to see so many individuals here with an interest in chronic fatigue syndrome (CFS) and this conference. I want to thank our co-chairs as well as the participants and observers.

For some time we have recognized that CFS, because it primarily affects women, is a disease that we could acknowledge and claim as a condition on which the Office of Research on Women's Health (ORWH) should focus. In 2001, the ORWH assumed the responsibility at the National Institutes of Health (NIH) for coordinating trans-NIH efforts related to CFS research. In addition, we brought on board Dr. Eleanor Hanna, who had been working with the Trans-NIH Working Group for Research on CFS, to become part of our office and to take the lead for this specific area.

The CFS Working Group represents an important part of our mission to encourage and stimulate interdisciplinary research. CFS is one of the scientific areas in which interdisciplinary collaboration will be vital to unlocking the essence of the condition. The CFS Working Group's initial effort in 2001 concentrated on preparing a program announcement to stimulate and broaden the scope of CFS research. That announcement was published in December 2002 and was based on recommendations from an ORWH-co-sponsored state-of-the-science conference held in October 2000.

In fiscal year 2002, the CFS Working Group focused its efforts on disseminating the program announcement and evaluating its effectiveness in developing a research plan and a specific request for applications to supplement that program announcement.

We want this workshop to help explore ways in which the neuroendocrine system acts as an intermediary between the brain and other body systems in explaining the diverse CFS symptoms. One goal is to determine whether or not these effects are primary or secondary in terms of central control in the brain. The chairs of today's sessions and Dr. Hanna will provide additional guidance about the goals of the conference and the specific scientific concepts to be addressed.

We look forward to the recommendations that you will bring forward. The Trans-NIH Working Group for Research on CFS and the Office of Research on Women's Health hope to develop and support an interdisciplinary request for applications, based on the underlying biologic mechanisms and questions identified at this scientific workshop. Your discussions and your contributions will be extremely important and will hopefully help us take the science to a different level—to help provide some of the answers for which those who suffer from CFS yearn.

We want this workshop to help explore ways in which the neuroendocrine system acts as an intermediary between the brain and other body systems in explaining the diverse CFS symptoms.

Welcome

Eleanor Hanna, Ph.D.
Office of Research on Women's Health

We are so fortunate that CFS is under Dr. Pinn's auspices. Not only has Dr. Pinn been a champion of women's health, but also she has made sure that underserved populations and under-recognized diseases are not forgotten at the National Institutes of Health.

I hope that today is the first of many meetings in which we gather an interdisciplinary group together, while we wait for answers to come from genomics and proteomics, and that we can begin to help people who have chronic fatigue syndrome and other unexplained medical illnesses. I thank you all in advance for your hard work during the next 2 days. I also hope you have fun!

Introduction

Leslie J. Crofford, M.D.

Welcome to all of the speakers and the meeting chairs, the NIH Coordinating Committee, Dr. Pinn, and all of you in the audience. This is a working meeting, so I am hoping that everyone in the room will join us in working hard to accomplish the goals set by Dr. Pinn to help the Trans-NIH Working Group for Research on chronic fatigue syndrome (CFS) develop a structure for future research on CFS, at least into one type of mechanism that may be operative: neuroimmune mechanisms and chronic fatigue syndrome. The principle question is, "Will the understanding of central mechanisms enhance the search for causes, consequences, and treatment of chronic fatigue syndrome?"

As laid out by the ORWH and the Trans-NIH Working Group, the purpose of this meeting is to enhance the scientific understanding of CFS by examining the interface between the brain, the immune system, and the symptoms of CFS and related disorders. In order to accomplish this, we needed to bring together basic and clinical scientists from diverse disciplines. Multidisciplinary description is the language of choice in discussing how to approach these multisymptom illnesses.

Why study the central nervous system (CNS) in CFS and related disorders? Although I have laid out some possible reasons to study the CNS, we all have to think broadly, with the help of the scientists in this room, to understand whether or not this is a reasonable approach.

The etiologies, or triggers and determinants, of susceptibility to CFS may be diverse. Some of the triggers—for example, chronic stress or genetic or environmental factors that mold the CNS—may alter CNS physiology. The principal symptom of CFS—fatigue—can be understood in terms of CNS physiology but may be related to diverse factors, including sleep. The effects of cytokines on the brain and cortical factors may be involved.

The questions to be explored in this workshop are:

- Can CFS and other related disorders be understood as disorders of CNS physiology?
- What methodologies are available to investigate disorders of CNS physiology?
- Are there therapeutic approaches that specifically target CNS physiology that should be applied to CFS?

We believe that the topics are relevant to CFS, but we need to keep an open mind as to whether or not we ought to be studying sleep, stress, and other realms of the CNS. We need to keep coming back to this question: What is CFS? We understand CFS in terms of its symptoms in people. As we listen to the basic science and the types of methodologies used, we need to keep coming back to the patients and the disorder—to understand how to apply to patients what is being discussed. We would like to get a sense from the workshop participants whether or not the areas we are discussing are important areas for research, and we hope to generate hypotheses for future study. The session chairs will summarize the sessions and the discussion, so we can all work together to generate hypotheses that we agree are useful for future research in CFS. The conference format is intended to be participatory, and we invite everyone to participate fully.

We need to keep coming back to the patients and the disorder—to understand how to apply to patients what is being discussed.

An Overview of Chronic Fatigue Syndrome

Dedra Buchwald, M.D.

This meeting is multidisciplinary and some participants may not be entirely familiar with all aspects of the definition of chronic fatigue syndrome (CFS) and previous hypotheses regarding its etiology. Therefore, my charge is to define the illness, review some of the prior research in terms of causative factors, and provide a larger context for its discussion.

What is CFS? CFS affects a minority of individuals who have disabling, long-term fatigue, and hence represents only a small part of the much larger world of fatigued individuals. CFS was first defined in 1994 by the Centers for Disease Control and Prevention, without an empiric basis, as a condition of debilitating fatigue enduring 6 months in the absence of any other disorders that could mimic CFS—for example, untreated hypothyroidism, cancer, rheumatoid arthritis, or psychiatric conditions that have been deemed exclusionary, such as bipolar illness. In addition, an individual must exhibit at least four of the following eight symptoms: problems with short-term memory or concentration, sore throat, lymph node tenderness, muscle pain, poor sleep, migratory noninflammatory joint pain, new headaches, and post-exertional fatigue. Those symptoms must have been present simultaneously for 6 months and must have started after the onset of the fatigue. This latter criterion was established because CFS is a syndrome, not a series of individual symptoms scattered over time. Lastly, it is notable that almost 90 percent of patients with CFS report that stress worsens their condition, 80 percent gain weight, and 75 percent need a daily nap; these symptoms are commonly reported but are not part of the case definition.

The fatigue itself also must have certain characteristics. The fatigue is required to have a definite onset and result in substantial reduction of occupational, educational, social, or personal activities. The fatigue cannot be attributable to exercise, insufficient sleep, or some other factor, or be completely relieved by rest. As noted previously, no medical or psychiatric conditions can be comorbid that might explain the fatigue. The CFS criteria also require that individuals undergo an evaluation that includes standard laboratory work—for example, a complete blood count, chemistry panel, and urinalysis—and a complete physical examination. However, no diagnostic test and no real markers for CFS exist; CFS is a clinical diagnosis of exclusion.

What causes CFS? The thinking in the field is quite diverse; therefore, the answer to "What causes CFS?" depends on who is asked. Most people believe that CFS has multiple causes and that it is a heterogeneous illness. The theories that have been postulated and investigated in the past 15 years reflect what patients complain about and the areas of expertise of investigators. A variety of different causes have been postulated. On one end of the spectrum, some investigators initially believed that CFS was actually not an illness at all but rather a societal problem of labeling, or that physicians were simply taking financial advantage of patients. On the other hand, others think that CFS involves multiple chemical sensitivities, and that sufferers are simply allergic to various environmental agents. Models of CFS must take into account expertise in various disciplines and link together research in many areas to better understand how previously reported abnormalities—or even normal findings—contribute to the pathophysiology of illness.

The answer to "What causes CFS?" depends on who is asked.

Research during the past 15 years was stimulated first by the observation that about 70 percent of people with CFS reported that their illness started with an acute viral or bacterial infection, particularly an upper respiratory or influenza-like illness, and that they never recovered. As a result, early

studies focused on viral agents, particularly viruses that had the ability to be persistent or latent, such as herpes viruses. Many viral and other microbial agents have been studied and then discarded as being the primary cause of CFS, although some studies have shown aberrations in antibody titers and subtle evidence of infectious agents.

The second wave of research focused on immunologic findings, triggered by CFS patients' reports of more frequent and severe illnesses and longer recovery times. The hypothesis was that if a primary viral or microbial agent did not directly cause CFS, then perhaps the immune system was not functioning properly. At least a dozen studies have examined various aspects of the humoral and cellular immune systems. For the most part, these studies observed decreased natural killer cell function, altered lymphocyte subset number and percent, and a different pattern of activation marker expression in people with CFS. Although these findings were not detected in all patients and all studies, they seemed to be more reproducible than other immunologic findings.

Any model of CFS must also take into account diverse published observations and findings.

The autonomic nervous system was the third major area of investigation. About 10 years ago, in the *Journal of the American Medical Association*, investigators from Johns Hopkins reported on their study of whether or not people with CFS were more likely than normal individuals to have a positive tilt table test. This test is used to diagnose neurally mediated hypotension, a form of autonomic nervous system dysfunction, by measuring blood pressure and pulse. Investigators found that a significantly greater number of people with CFS had a positive tilt table test than did healthy controls. Other research on the autonomic nervous system has focused on altered heart rate variability. Many of the studies of the autonomic nervous system have been conducted with children. Since investigations among adults have not consistently replicated the original findings, it remains unclear how much of a role the autonomic nervous system plays in CFS.

A fourth area of inquiry has been objective measures of sleep. Virtually all CFS patients have subjective sleep problems. On polysomnography, CFS patients sleep less efficiently, spend more time awake after going to bed, and have altered stages of sleep. In most studies, CFS patients have normal REM (rapid eye movement) latency, thus differentiating their sleep from the sleep of patients who have major depression.

Recently, a fifth topic of investigation has asked whether or not a genetic predisposition exists for fatigue in general, and more specifically for CFS. In a study of 146 pairs of female twins, we found that the concordance rate for a CFS-like illness among identical twins was 38 percent, but 11 percent for fraternal twins.[1] From this finding we infer that there is a familial, if not truly genetic, predisposition to fatiguing illnesses. Several other studies in the literature support the observation of a genetic predisposition to fatiguing illnesses.

Lastly, a theory that has permeated the literature since CFS was first described attributes CFS to some form of atypical depression or another psychiatric disorder that has yet to be well defined. The basis for this hypothesis was the observation that rates of psychiatric disorders among CFS patients are three or four times higher than those in the general population. However, many people with CFS do not have a current or lifetime diagnosis; thus, this model is not fully explanatory. In addition, a lifetime disorder does not explain a current symptom or illness. Nevertheless, although psychiatric illness does not directly cause CFS, the high prevalences of psychiatric illnesses must be part of any model of CFS. In this regard, research on cortisol and ACTH (adrenocorticotrophic hormone) has demonstrated that both the levels and the type of responses were different in CFS patients from those in individuals diagnosed with major depression.

Any model of CFS must also take into account diverse published observations and findings. For instance, some experts believe that CFS is part of a family of disorders and that it is not a unique, identifiable disorder. Many demographic and clinical aspects of conditions such as fibromyalgia, irritable bowel syndrome, and temporomandibular disorder are similar to those of CFS, although controversy exists about the usefulness of thinking about them as the same condition. Regardless, CFS models must explain the fact that these conditions overlap to some extent.

Additionally, studies have detected a variety of biological abnormalities that are not specific for CFS and that are not consistently associated with severity

or type of symptoms. Most of the findings have been described in isolation from other findings and without reference symptoms or clinical status. Nonetheless, a model of CFS must explain such findings.

A final issue that investigators will need to address in designing studies that elucidate a model for CFS is the many meanings of the word "fatigue." Fatigue is a broad term that should be defined with more specification. Many kinds of fatigue exist. Fatigue has often been classified as physical fatigue and mental fatigue, as distinguished from weakness and sleepiness, which are distinct phenomena. Of note, a large study of fatigue in primary care in the United Kingdom involving 33 million patient years of data has documented 69 synonyms for fatigue, thereby demonstrating its multifaceted and heterogeneous nature (Peter White, personal communication, 2003). Fatigue results from not having enough sleep, jet lag, physical activity, losing a loved one, and being depressed. For example, if you lose a loved one, you become depressed and fatigued, but over time you get better. What is the physiologic core of recovering? One model for CFS that directly examines the central role of fatigue is that persons with CFS "get off the road and into the ditch" and cannot get back on the road again. An intriguing approach to CFS would be to examine how normal people recover from fatigue.

Sleep medicine can discriminate between the physiology of sleepiness and tiredness related to physical exhaustion. The Multiple Sleep Latency Test consists of a series of 20-minute nap opportunities used to determine how quickly a person falls asleep, thus providing an indication of sleepiness. We know almost nothing about the physiology of fatigue. We can say it is not sleepiness, but we cannot differentiate types of fatigue experienced as a response to an adverse task, grieving, or simple exhaustion. No studies address the physiology of fatigue in pregnancy, although 40 to 50 percent of women experience considerable fatigue during the first trimester. This dearth of studies underscores that we know virtually nothing about perhaps the single most common class of fatigue among women.

Is neuroendocrine dysfunction the cause or the result of physical inactivity?

Many researchers believe that the pathophysiology of CFS is not well understood, but most symptoms and findings can be explained by neuroendocrine aberrations. We need to focus on models that can link mechanism and causation, and studies should be designed for this purpose. However, any explanatory or conceptual model of CFS also must explain diverse observations such as the following:

- *Female predominance*. In most studies, CFS affects women more often than men.
- *Acute onset*. Most patients describe the onset of this illness as occurring over hours or days.
- *Uniformity of symptoms*. Most people with CFS report poor sleep, high stress levels, an inability to react to stress, pain, psychological distress, and a variety of other symptoms.
- *Relative absence of prominent findings on physical examination*. Most CFS patients do not have objective evidence of an inflamed throat, enlarged lymph nodes, or muscle weakness.
- *A variety of objective findings across multiple domains*. These findings are present in some but not all people.
- *Substantial disability*. Despite the lack of findings on physical examination and lack of a diagnostic marker, this illness causes substantial disability in most people.
- *Altered perception*. The literature suggests that persons with CFS experience an altered perception of effort pertaining to sleep, exercise, cognition, and across a variety of other domains.

How can we build a model of CFS? We must think outside the box and, along with conducting traditional investigations, we must use innovative, creative study designs and new paradigms, and we must look to other fields for inspiration, new collaborators, and other experts for guidance.

What are some examples of investigations that contribute to a model for CFS? Using a model in which neuroendocrine dysfunction is the primary aberration in CFS, one question might be, "Is neuroendocrine dysfunction the cause or the result of physical inactivity?" One way to answer that question is to conduct a case control study that compares the neuroendocrine status of CFS patients with healthy, extremely sedentary individuals, such as those in traction. Another model-building study would address the observation that women get CFS more often than men. Questions of interest might be whether female reproductive hormones play a causative role in CFS or whether use of exogenous hormones increases the risk for CFS. These questions could be answered by using epidemiologic data to

examine the prevalence of CFS in women who are taking oral contraceptives or hormone replacement therapy compared to those who are not.

A third example would be to study the biological underpinnings of perception and its alteration in CFS. One question might be whether there are objective measures of perception that quantify the subjective patient reports of heightened sensitivity to noise, visual disturbances, and paresthesias. Scientific investigations using models of disease have tried to answer these kinds of questions in other conditions. For example, a case control study was conducted among students, half of whom were sent to a discotheque and were exposed to loud noises for 6 hours. Subsequently, all the students were put in a quiet environment and investigators were able to link biological markers to reports of sensitivity to noise. In a similar approach, researchers could compare visual acuity, urinary catecholamines, and cortisol in CFS patients and control groups in quiet and stimulated conditions. However, such studies have not been performed for CFS.

Another interesting paradigm and an example of an innovative methodology used the tickle response to link external stimuli with internal processing. This model also might shed some light on how CFS patients process information and stimuli. Since the time of Aristotle, it has been common knowledge that you cannot tickle yourself; even though the motor movement or stimulus is the same, someone else has to tickle you to elicit the tickle sensation. In one study, investigators tickled patients and then the patients tickled themselves. MRI scans showed that when patients tickled themselves, the somatosensory cortex did not light up but the cerebellum did; the opposite occurred when another person tickled them. The authors concluded that the tickle response visibly affects the somatosensory cortex if another person elicits it. However, the absence of predictability makes a person perceive a stimulus as ticklish and the cerebellum mediates predictability. Investigators then conducted a second experiment in which they told patients that a machine was tickling them. By indicating that the machine was going to tickle them, they were able to prove that it was the element of unpredictability that made a tickle ticklish. That series of clever experiments examined how people perceive and process information. We need to describe how CFS patients perceive and process information, and how this differs from healthy people, to better understand this illness.

The challenge is to use this meeting's collective expertise to articulate a testable explanatory model for CFS that focuses on the neuroimmune, hormonal, and other central nervous system processes in CFS. This model should include elements from multiple fields and many domains and disciplines and should lead to an understanding of the relationship between objective, scientific, and diagnostic findings and the symptoms and suffering experienced by CFS patients.

Reference

1. Buchwald D, Herrell R, Hartman S, Belcourt M, Schmaling K, Goldberg J. A twin study of chronic fatigue. Psychosom Med 2001;63:936-943.

The challenge is to use this meeting's collective expertise to articulate a testable explanatory model for CFS that focuses on the neuroimmune, hormonal, and other central nervous system processes in CFS.

Chair: Leslie J. Crofford, M.D.

Health Consequences of a Dysregulated Stress Response

Esther M. Sternberg, M.D.

The stress response includes two main arms: the neuroendocrine stress response and the adrenergic sympathetic nervous system response. The neuroendocrine stress response includes the hormones of the hypothalamic-pituitary-adrenal (HPA) axis. The hypothalamus produces corticotropin-releasing hormone (CRH), which is the brain stress hormone; the pituitary gland produces adrenocorticotropic hormone (ACTH); and the adrenal glands produce glucocorticoids. The sympathetic nervous system is also activated simultaneously during the stress response. The peripheral nervous system is another component of the central nervous system that plays an important role in regulating immune responses.

Disease results when the regulatory negative feedback loop of the CNS on the immune system is out of control. Too much activation of the brain's hormonal stress response tunes down the immune system and can lead to infection. Too little activation can predispose the body to inflammation, because the endpoints of the HPA axis on the immune system are the glucocorticoid hormones, which are usually immunosuppressive. However, at lower physiological concentrations, glucocorticoid hormones can also be immunostimulatory.

Glucocorticoids regulate immune responses, not simply because they are drugs but because they are the endpoint of the brain's sensitive hormonal stress response. This hormonal stress response is set to respond moment by moment to every change in the environment. People instantaneously respond to environmental stimuli, or stressors. These changes affect the immune system through the hormonal cascade, and ultimately through the glucocorticoid receptor that is part of a family of nuclear hormone receptors. The glucocorticoid receptors sit in the cytosol, the fluid portion of a cell's cytoplasm, and bind to glucocorticoids. When the hormone binds to the receptor, it allows the activated receptor to dimerize, move to the nucleus, and facilitate translation and transcription of proteins.

Chronic stress is a biological event that results in dysregulation of the brain's hormonal stress response. Conditions that have been shown to be associated with chronic stress include prolonged wound healing (based on studies done by Janice Kiecolt-Glaser and Ronald Glaser at Ohio State University), increased severity and incidence of viral infection (studies by Sheldon Cohen and colleagues), and decreased antibody production to vaccine (studies by John Sheridan and colleagues and Kiecolt-Glaser and colleagues). These sorts of effects have been shown in chronic caregivers of patients with Alzheimer's disease, in situations of marital stress, in students undergoing exam stress, and in Army Rangers undergoing extremes of exercise.

In the case of too little hormonal stress response (if the HPA axis is not active enough), the opposite result occurs: the immune system is tuned up and the external hormonal brake to stop out-of-control inflammation no longer exists. When producing too little CRH, too little ACTH, and too little glucocorticoids, and when exposed to an antigenic or pro-inflammatory stimulus, immune cells no longer

have a shut-off valve from glucocorticoids. Under such conditions, the individual may develop autoimmune inflammatory disease. For example, the HPA axis hypo-responsive Lewis rats develop many autoimmune inflammatory diseases, with varying patterns according to the pro-inflammatory stimulus to which the rat was exposed. If not exposed to such environmental inflammatory triggers, Lewis rats live long, healthy lives with no autoimmune inflammatory disease. However, when Lewis rats are exposed to streptococcal cell walls, lactobacillus cell walls, mycobacterial cell walls, or other bacteria, they develop an inflammatory autoimmune disease that looks like rheumatoid arthritis. Similar results are seen when Lewis rats are given collagen and adjuvant arthritis. When exposed to extract of spinal cord with myelin basic protein, they develop a multiple sclerosis-like disease called experimental allergic encephalomyelitis (EAE). Depending on the nature of the pro-inflammatory trigger to which they are exposed, Lewis rats can develop autoimmune myasthenia gravis, experimental sialadenitis, experimental autoimmune thyroiditis, adrenalitis, uveitis, orchitis, myocarditis, or nephritis. Thus, many animals with a predisposition to autoimmune disease will not develop disease until they are exposed to an environmental trigger, and the nature, dose, and route of exposure all contribute to development of disease.

A blunted HPA axis is only one factor determining whether an individual animal or organism will develop chronic autoimmune inflammatory disease after exposure to an antigenic or pro-inflammatory stimulus. Other factors, including genetic predispositions and other hormonal factors, play an important role in the development of autoimmune disease. Sex hormones, their receptors, and the hypothalamic pituitary gonadal axis all play a critical role in the development of autoimmune inflammatory diseases. Thus, females of most species have a higher incidence and/or severity of most autoimmune inflammatory diseases. For example, female-to-male ratios are 10:1 for lupus, 2:1 for rheumatoid arthritis, and in the 2:1 to 10:1 range for other similar diseases. Autoimmune diseases are multigenic and polygenic—many genes control them, each with a small effect. More than 20 different regions on 15 different chromosomes determine susceptibility and resistance to inflammatory arthritis.

Fischer rats are closely related to Lewis rats; about 66 percent match across the genome, and the two strains are histocompatible. However, Fischer rats are resistant to developing the same autoimmune diseases in response to the same dose, route, and type of pro-inflammatory triggers. Early studies found a high inflammatory response to carrageenan, an innate trigger, in Lewis rats and virtually no such response in Fischer rats. Lewis rats exhibit blunted corticosterone, ACTH, and CRH responses, compared to Fischer rats that show a hyper response in all cases.

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Similarly, these two strains of rats behave differently in response to stress. This is not surprising: CRH is not only a neuroendocrine hormone that acts peripherally on the immune and other systems in the body, but it is also a neuropeptide that is expressed throughout the brain and plays an important role in stress behaviors. A Lewis rat placed in the novel environment of an open field will run around and explore in a relaxed manner; a Fischer rat in a novel environment will exhibit stress behaviors, including decreased locomotion, freezing, grooming, and rearing. These findings do not mean that one rat strain is less stressed than the other or that they are exposed to different amounts of stress. The findings mean that the rats are more or less responsive to a stressful situation, most likely in part because of the difference in stress hormone levels in the brains.

The concept of an association between a blunted HPA axis response and susceptibility to autoimmune disease crosses species and diseases. A blunted HPA axis response is shown by chickens with thyroiditis and scleroderma; some strains of mice with lupus; rats with other illnesses besides arthritis such as experimental allergic encephalomyelitis, septic shock, inflammation, and others; and humans with rheumatoid arthritis, lupus, Sjögren's syndrome, dermatitis, asthma, fibromyalgia, CFS, and irritable bowel syndrome.

Clinical studies of Sjögren's syndrome, lupus, dermatitis, and asthma have found that the blunting may be present at different points in the HPA axis or with different patterns, but the dysregulation of the axis exists in all these conditions. Without being exposed to a stressful stimulus, the differences between the two strains of rats—or any two humans—are not evident. The response is individual, but the stress hormone axis must be stimulated in order to distinguish among the high and low responders. The axis can be turned on with administration of hormones of the stress response, such as intravenous ovine CRH, by inducing hypoglycemia, or with psychological stressors, such as public speaking and mental arithmetic.

Genetic interruptions of the HPA axis (as in Lewis rats), surgical interruption in animal models of the axis (by removing the pituitary or the adrenals), or pharmacological interruptions (by blocking the glucocorticoid receptor with drugs) all tend to predispose to worse inflammatory disease. Even in inflammatory-resistant strains, such as Fischer rats, when the axis is blocked pharmacologically or surgically, the inflammatory-resistant animal will become so susceptible to inflammation that it will die within the first 12 hours of exposure to a pro-inflammatory stimulus, such as bacterial cell walls.

The concept of an association between a blunted HPA axis response and susceptibility to autoimmune disease crosses species and diseases.

We demonstrated this effect using RU486, a progesterone receptor antagonist and also an equally potent glucocorticoid receptor antagonist. Neither RU486 alone nor streptococcal cell walls alone have any effect in Fischer rats. Combined, however, some of the rats died rapidly in septic shock and the surviving animals developed arthritis. The effect was reversed with corticosterone. Other researchers have shown similar effects in otherwise inflammatory disease-resistant animals whose HPA axis was interrupted by adrenalectomy, hypophysectomy, or treatment with glucocorticoid receptor antagonists. In these studies, ordinarily resistant animals with EAE, or those exposed to salmonella, Shiga toxin, or murine cytomegalovirus developed shock with a high incidence of mortality. These animal studies demonstrate that if the HPA axis is blocked at any point and in any way (at the level of the adrenals, the pituitary gland, or the glucocorticoid receptor), an inflammatory-resistant animal will become highly susceptible to inflammation and potentially to the lethal effects of shock. Reconstituting the axis with glucocorticoids or by hypothalamic transplants, as in studies in Lewis rats, can reverse the process. In this latter case, inflammatory-susceptible Lewis rats became resistant to inflammatory disease when they received Fischer hypothalamic transplants, at the same time that their HPA axis responsiveness was corrected.

Researchers have also studied why Lewis rats have a blunted CRH response. A number of neuropeptide/neurotransmitter systems are differentially regulated in inflammatory-resistant and inflammatory-susceptible rats. It is difficult to discern which is cause and which is effect, but multiple systems that regulate the immune system are dysregulated. CRH is blunted, but so is the sympathetic nervous system response.

Studies conducted a number of years ago looked at noradrenergic responses in Lewis and Fischer rats in response to hypoglycemia. A blunted sympathoneuronal response was observed in the Lewis rats compared to the Fischer rats, suggesting the following important issues:

- The sympathetic nervous system innervates immune organs such as the spleen. Spleen cells are in close apposition to the noradrenergic nerve terminals. There is regional regulation of immune organs—such as the lymph nodes, spleen, and thymus—by the sympathetic nervous system.
- Neuroanatomically, investigators want to know why there is a dual dysregulation of the HPA axis—CRH in the hypothalamus and sympathetic responses. Dr. Sternberg's studies have looked at the distribution of CRH mRNA in the two strains of rats and have found that it is not that the Lewis rats do not make any of the brain stress hormones, but rather that they make it in different distributions. A three-dimensional reconstruction of serial sections in the Lewis rats shows a part missing paraventricular nucleus of the hypothalamus, an expression of CRH in the part of the brain

that projects to the autonomic nervous system. It is possible that in Lewis rats there is a dual blunting of these two important arms of the hormonal stress response and the neuronal stress response that may be related to some regional cellular dysfunction at the level of neurons that connect the two systems within the brain. This theory merits further explanation.

Complex autoimmune inflammatory diseases are regulated by many genes, each with small effect—thus they are polygenic and multigenic. Genetic linkage and segregation studies in Lewis and Fischer rats showed two regions on two different chromosomes that link to the inflammatory susceptibility and resistance of these two strains of rats. These 2 regions exactly superimposed on 2 of the more than 20 different regions on 15 different chromosomes that many other researchers have shown to be associated with inflammatory arthritis. These findings have important implications in complex illnesses such as CFS. The familial tendency of CFS is well known. If an individual inherits all 20 of the regions associated with inflammatory susceptibility, as do Lewis rats, there is a high probability that, like the rats, this individual will get an inflammatory disease when exposed to inflammatory triggers in the environment. If, on the other hand, like a Fischer rat, a person inherits few of the susceptibility regions or inherits inflammatory-resistance genes, she or he is far less likely to contract an inflammatory disease, even when exposed to inflammatory triggers. Thus, in complex diseases, it is important to remember that there are many regions on many chromosomes that contain many genes that predispose to these kinds of illnesses, and there is a genetic dose effect of the number of these regions inherited.

The next question to explore is what candidate genes are in these linkage regions. Rat chromosome 10 is identical to a region on human chromosome 17 that links to many autoimmune diseases. It contains a number of genes that regulate both the neuroendocrine stress response and inflammation. Looking at two of these genes, we found no difference in the coding region on the CRH type 1 receptor and believe there also is no difference in the promoter region, because there is no difference in regulation of these receptors in the two strains. Angiotensin-converting enzyme is another gene present in the chromosome 10 linkage region that regulates both CRH and inflammation, but it is unlikely that that mutation contributes to the trait.

The important message of these studies is that the genetic contribution to the variance in the inflammatory trait in this model is low (approximately 35 percent), compared to the environmental contribution (approximately 65 percent). Similarly, in conditions such as diabetes, cardiovascular disease, hypertension, inflammatory arthritis, lupus, and multiple sclerosis, the variance in the trait contributed by genotypic factors typically is only between 35 and 40 percent. For the geneticist, this means it will be difficult to find the gene or genes that predispose to these traits. However, newer technologies are now available to examine many potential candidate genes simultaneously. We looked at gene expression microarrays to determine how patterns of gene expression may differ in different individuals or groups. Studying the Lewis and Fischer rats exposed to the pro-inflammatory stimulus lipopolysaccharide (LPS), we surprisingly found few genetic differences—only five genes that are differentially expressed in the hypothalamus in the two strains of rats. Three of the genes are unknown expressed sequence tags. The other two are transthyretin (TTR), a thyroid hormone transporter, and cholecystokinin (CCK), both of interest in this context. Future studies will look at whether differential thyroid hormone axis regulation could explain some of the differential CRH and HPA axis responsiveness differences in these strains, a complicated investigation because it is difficult to know whether the HPA axis change is predisposed to the thyroid hormone changes or vice versa.

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When considering CFS, the finding of differential TTR is of interest because people who have hypothyroidism experience fatigue. Perhaps an area for future exploration should be interactions between multiple

hormone axes, at least examining axes with secondary roles that could be amplifying or contributing to a problem.

The finding that only 35 percent of the variance comes from genetic factors and 65 percent comes from environmental factors can be viewed as a positive finding for implications in managing complex diseases such as arthritis, because environment can be more easily controlled than genetics. One environmental factor that has been studied is maternal behavior and maternal-pup interactions in rats. In examining maternal behavior, we found that Lewis rats with a blunted HPA axis response had a rapid retrieval rate of their pups, while the Fischer rats completely ignored the pups for the entire 15 minutes of the test time in which pups are separated from the mother around the edge of the cage. The Lewis mothers picked up their pups, put them in a corner, crouched, and nursed them within 3 minutes of separation while the Fischer mothers groomed themselves and ran around, ignoring the pups and displaying stress behavior. Cross-fostering studies, where mothers of the different strains adopt and raise the opposite pups indicate that strain, gender, and maternal environment interactions determine adult stress responsiveness in this model.

If this happens in the wild or in the laboratory setting, what happens to the offspring? During the past 25 years, studies have found that separation of rat pups from their mothers for as little as 5 to 15 minutes a day for the first 2 weeks of life result in a permanent increase in HPA axis responsiveness that lasts through adulthood. Many factors contribute to this increase. Some involve temperature, but even when researchers control for temperature and thyroid hormone responses, animals still were left with a permanent change in the stress axis. Thus, the stress hormone axis is plastic, and there are critical periods in development when it can be changed. What is not known is whether there are also critical periods in adulthood when the responsiveness of the stress axis can be changed, how much it might be changed in adulthood if at all, and exactly how these animal findings can be extrapolated to humans. It is known from human studies in adopted Romanian orphans who experienced extremes of deprivation, malnutrition, and illness in early infancy that adoption into Canadian families before the age of 9 months was associated with relatively few sequelae, whereas some deleterious effects were seen in infants adopted after the age of 9 months. It is likely, therefore, that in humans far greater degrees of early environmental stress may be required to permanently affect the stress hormone axis than in rodent models. Nonetheless, these are areas of research that need to be addressed to understand factors contributing to dysregulated stress hormone responses in complex illnesses, including arthritis and CFS.

Another level at which the stress hormone axis can be dysregulated is at the level of the glucocorticoid receptor. In rheumatoid arthritis, and to a lesser extent in lupus, there are glucocorticoid receptor polymorphisms in the glucocorticoid receptor-beta, which is thought to be a dummy form of the glucocorticoid receptor that could act as an endogenous glucocorticoid receptor antagonist. The receptor has been shown to be elevated in several autoimmune, inflammatory, and allergic diseases (e.g., irritable bowel syndrome and asthma). This mutation is associated with increased receptor mRNA stability and increased receptor levels. Alternatively, it is possible that in some individuals with these illnesses, problems may occur at the level of the hypothalamus or sympathetic nervous system as in Lewis rats, in the pituitary, the adrenals, or the glucocorticoid receptor. In all cases, some degree of glucocorticoid resistance would result and, wherever the interruption occurs, it will predispose an individual to enhanced susceptibility and severity of inflammatory disease when the individual is exposed to inflammatory triggers, because of a lack of anti-inflammatory, immunosuppressive effects of glucocorticoids.

In summary, these illnesses must be considered at multiple levels. Multiple genetic factors predispose the body to these illnesses. Potentially, developmental factors can contribute to the onset of illnesses. Environmental exposures are also probably necessary to trigger such illnesses. The hosts' responses to all of these factors depend on the set point of multiple host response systems. This applies to neural (autonomic) responses, the neuroendocrine (HPA, hypothalamic-pituitary-thyroid [HPT]) response systems, and the sex hormones (hypothalamic-pituitary-gonadal [HPG] axis). After an

individual is exposed to environmental factors—viral, bacterial, or inflammatory—all of these hormonal and neural factors together contribute to determine whether an individual recovers quickly or has persistent symptoms. In studying CFS, it will be critical to focus not only on environmental triggers but also on the resiliency of the host's response systems in returning to baseline. Everyone is exposed to triggers, but some individuals' response systems quickly return to baseline while others take a much longer time or never completely recover, and it may be this factor as much as the nature of the initial response that determines the ultimate outcome of a disease.

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Stress and the Augmentation of Immune Function

Firdaus S. Dhabhar, Ph.D.

About 4,500 years ago, the Nei Ching, the ancient Chinese classic on internal medicine, listed nine groups of disease-causing agents. The list included the emotions as potential causative factors in disease, stating that worry and imagination can upset the functioning of the spleen. In many ways, this sums up what the field of psychoneuroimmunology is trying to understand today. Wondering how different mental and emotional states can affect health is not a monopoly of the ancients. Cover articles of many popular magazines today deal with stress, indicating how much this subject occupies modern thinking.

Stress can be defined as a constellation of events that begins with a stimulus (the stressor), which precipitates a reaction in the brain (stress perception), which then results in the activation of fight-or-flight systems in the body (the physiological stress response). Stress is defined in the following broad categories:

- Acute stress lasts for minutes to hours in duration.
- Chronic stress persists for weeks, months, or years and involves a dysregulation of the circadian rhythms, leading to well-known deleterious consequences.[1]

The activity of the hypothalamic-pituitary-adrenal (HPA) axis is one example of a psychophysiological stress response. A stressor could be a prototypical danger stimulus, but it could also be a prototypical excitatory stimulus, such as a first kiss on a first date, which may not often be regarded as a stressor but which often activates the same types of stress axes. A stress perception in the brain results in a physiological stress response. The HPA axis response results in the ultimate elaboration of glucocorticoid hormones that signal to the rest of the body that something is happening or is about to happen.

The HPA axis is not the only stress response system. Many other systems involve the release of epinephrine, norepinephrine, endogenous opioids, prolactin, cytokines, and other hormones during a physiological stress response. (Most of the work from our laboratory has been mediated by epinephrine and cortisol.) While it is possible that other hormones contribute to these effects, none have yet been found to equal the significance of cortisol and epinephrine.

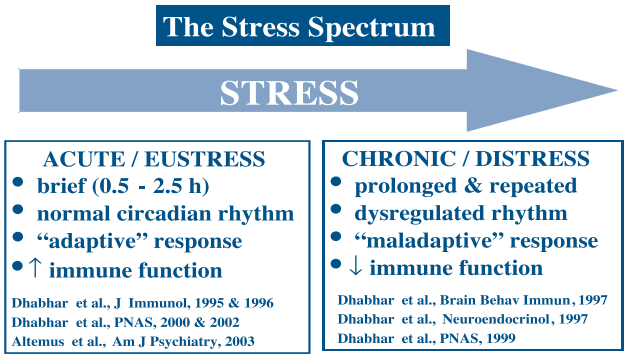
A poster from the National Library of Medicine describes stress as "a loaded gun," and goes on to state, "If left untreated, stress can kill you, just as surely as a bullet. Do not wait for the gun to go off. Get help today." There is validity to this claim, but there are other ways to consider it. First, it need not necessarily or always be the case that stress is harmful. Second, while a loaded gun can kill if used unwisely or if in the hands of an enemy, a loaded gun can also protect if it is used effectively.

Just as the stress response prepares the cardiovascular, musculoskeletal, and neuroendocrine systems for fight-or-flight, it may also prepare the immune system for challenges, such as wounding or infection, that may be imposed by the actions of a stressor. In other words, short-term stressors may enhance immune function within compartments, such as the skin, that are likely to be compromised by the actions of a stressor, such as a predator.[2] Without a full-blown efficient psychophysiological stress response, a predator has little chance of capturing its prey and hence feeding itself and surviving. Similarly, without a full-blown psychophysiological stress response, the prey has little chance of surviving and escaping the predator. An efficient psychophysiological stress response gives both predator and prey the chance to survive. This leads to the fundamental principle that the psychophysiological stress response is a crucial evolutionarily conserved survival system.

The central goals of the research in my laboratory are:

- To identify the cellular, molecular, and physiological mediators of nature's survival systems, the successful stress response.
- To harness these mediators to maintain health or to restore health in case of disease.
- To figure out ways to intervene in situations in which nature's survival system may itself contribute to pathology.

To reconcile the different views of stress, stress can be viewed as a spectrum of possibilities. At one end of the spectrum is acute stress, what Hans Selye labeled "eustress." Eustress involves brief duration stressors of 1/2 to 2 1/2 hours and a normal circadian rhythm. It is an adaptive response and involves an enhancement of immune function in certain compartments. At the other end of the spectrum is chronic stress, or distress, which is prolonged or repeated in nature, involves a dysregulated circadian cortisol rhythm, is a maladaptive response, and is well known to suppress immune function. Many researchers have looked at this aspect of chronic stress and immune suppression. Of interest is the part of the spectrum representing resilience, as it takes a significant amount of loading on biological systems before they move from the adaptive end of the spectrum to the pathological end.



Adapted from: Dhabhar & McEwen, *Brain Behav Immun*, 1997

We use restraint, novelty, and social conflict as stressors in our animal studies. These are naturalistic, brief, non-painful, non-severe stressors that generate physiological stress responses. In clinical studies, the process of undergoing surgery (knee and oral) is used as a stressor as well as the Trier Social Stress Test (TSST), in which subjects are challenged with mental arithmetic problems and public speaking. The goal of these experimental models is to simulate the fight-or-flight stress response. Results of one TSST study that examined hormones in the bloodstream after arithmetic and public speaking challenges found that different people mount different degrees of stress response. In one subject, for example, there was an ACTH response in the plasma about 20 minutes after the challenge, a cortisol response, and a norepinephrine response.

In animal and human studies, what happens to immune cell numbers in the bloodstream during acute stress? Understanding of the meaning of these changes remains preliminary. What is seen is a redistribution of the body's "soldiers," or immune cells, during acute stress, so that these soldiers are moving from their barracks into the boulevards and onto battle stations.[3,4] These military analogies mean that in the early phases of a stress response, immune cells mobilize from "barracks"—organs such as the spleen and the bone marrow—into the bloodstream (the boulevards), registering as an increase in lymphocytes and monocytes in the blood. As the stressor progresses, there is a decrease in lymphocyte and monocyte numbers in the bloodstream, which was once perceived as a net loss of cells in the body. More than 50 percent of the circulating immune system is lost during acute stress, which is one of the reasons this type of stress was once thought to be immunosuppressive. However, researchers have found that this decrease in immune cells in the blood does not represent a net loss of cells from the body, but rather redistribution of cells from the bloodstream to organs such as the skin and the lymph nodes, and perhaps the gastrointestinal and urogenital tracts. The skin and lymph nodes have been identified as critical targets—the battle stations—to which leukocytes are known to traffic during an acute stress response. Neutrophils behave slightly differently than lymphocytes and monocytes, but still in agreement with the central hypothesis.

In examining functional consequences of this redistribution of leukocytes to the skin and skin-draining sentinel lymph nodes during acute stress, researchers are looking at whether this activity increases the ability of an organism to mount immune responses in the skin following an acute stress response. Several different animal models, as well as human studies, are used to test functional consequences. A wound-healing model and a cell-mediated immunity model are being studied in humans and animals.

The cell-mediated immune model enables researchers to tackle different angles of these functional consequences. The hypothesis being tested is that acute stress experience before antigen exposure will enhance the ensuing immune response. To test this hypothesis, the skins of control and acutely stressed animals were exposed to antigen and then examined for differences in immune response. An increase in pinna thickness, a measure of a gross integrated immune response much like a physician measuring the increase in diameter of a tuberculin skin test, was seen in a group of non-stressed animals whose skin was exposed to an antigen. The animals mounted a prototypical immune response that peaked at about 24 to 48 hours, similar to a classic poison ivy delayed-type hyposensitivity response. A cross-section of skin exposes the stratum corneum, epidermis, dermis, striated muscle, and cartilage. A sample taken 24 hours post-stress exposure revealed a massive leukocyte infiltration (visible as blue dots with a hematoxylin eosin stain) moving to the stratum corneum, as predicted, because the antigen was administered on the top surface of the skin.[5] The infiltration peaked about 24 to 48 hours after antigen administration.

The immune response from animals that were stressed a single time for 2 hours before an antigen was placed on their skin occurred at a faster rate, attained a higher peak, and remained significantly elevated compared to the immune response of the non-stressed animals. Histological sections compared at exactly the same time showed a much more vigorous immune response in the animals that were acutely stressed before receiving the antigen, with a significantly larger number of leukocytes and distention or swelling of tissue.[5] Other immunocytochemical markers indicated not only that more cells were coming into the site of the acutely stressed animal that had been challenged, but also that these cells showed a more activated profile, as measured by some of their activation markers.

This response has been observed in a number of different models, including mice, hamsters, rats, and nonhuman primates, and in some human studies. In a study of patients with post-traumatic stress disorder (PTSD) due to childhood abuse, we found enhanced delayed-type hypersensitivity (DTH) response, as in the animal model.[6] Several potential mechanisms are being explored to mediate this effect, including enhanced acute stress reactivity that PTSD patients are known to have. Other potential mechanisms are enhanced levels of pro-inflammatory cytokines that PTSD subjects often show and decreased levels of basal cortisol that have been observed in some PTSD cases. In this study, no differences in basal cortisol levels between controls and PTSD subjects were seen. The implication of this study may be that the enhanced immune reactivity seen in PTSD plays a part in mediating the greater number and frequency of inflammatory disorders reported by PTSD patients.

Whereas acute stress enhances immunity, chronic stress suppresses the immune response.

A number of other studies that examined infection, wounding, and antigen-specific responses concluded that acute stress enhances both innate (primary) immune responses and adaptive (secondary) immune responses. The mediators of the immuno-enhancement are physiological concentrations of epinephrine and corticosterone, which are mediating the immuno-enhancing effects of acute stress.

Stress-induced enhancement of immune function can be protective, as in the case of wound healing or resistance to infection. However, stress-induced enhancement of immune function can also have pathological effects. Examples are cases of exacerbating allergic or inflammatory reactions of autoimmune diseases, which might include CFS. Many CFS patients say stress worsens their condition. If there is an inflammatory etiology to CFS, it is possible that it might be part of the mechanism by which stress exacerbates some of the inflammatory components of the syndrome, thereby worsening symptoms.

We have also tested the hypothesis that, whereas acute stress enhances immunity, chronic stress suppresses the immune response. Several studies have confirmed this hypothesis. In one study of chronically stressed animals, skin-cell-mediated immune response time was slower than in a control group; in another, chronic stress administered before antigen exposure significantly suppressed the immune response.

Circadian rhythms also play a role. A Swahili proverb states: “Every morning a lion wakes up knowing that it has to outrun the slowest gazelle or it will starve. Every morning a gazelle wakes up knowing that it has to outrun the fastest lion or it will die.” The moral of the story is that whether one is a gazelle or a lion or even a human, one must be ready to run every morning. The relevance to the current discussion is the relation of circadian rhythms to the immune

system. An internal alarm clock gets people—and lions and gazelles—ready to run every morning. This alarm clock taps into the same hormonal access as the stress axis. Just as activating the HPA axis during stress can trigger cortisol secretion, the HPA axis is also influenced by the circadian rhythm. At the beginning of the active period of the diurnal cycle, there is an increase in cortisol or corticosterone levels, and near the end of the active period of the diurnal cycle there is a decrease. This presents a natural phenomenon against which to test findings with acute stress.

Based on acute stress studies, we predicted that blood lymphocyte and monocyte numbers would decrease when corticosterone levels increased, that skin leukocyte numbers would go up at the same time (as they do with acute stress), and that a skin immune response would show enhancement if the animals or humans were challenged on the skin at the beginning of the active period of the diurnal cycle. Real time data of the corticosterone rhythm found that when corticosterone levels rose at the beginning of the active period, leukocyte numbers fell. When corticosterone levels fell, leukocyte numbers rose, a result that has been reported by many researchers.

Another prediction, based on the acute stress studies, was that challenging the skin of an organism at the beginning of the active period of the diurnal cycle, when leukocyte numbers in the blood are lowest, would find leukocyte numbers in the skin at their highest, with a larger immune response for an active period challenge.

In the Stanford breast cancer studies, some subgroups of patients showed normal circadian cortisol rhythms and some were dysregulated. In the normal rhythm patients, cortisol was high in the morning at the beginning of the active period and low in the evening. In the dysregulated patients, cortisol was basically flat, starting out high in the morning and staying high through the evening. The dysregulated diurnal cortisol rhythm appeared to be correlated with increased mortality in these patients, pointing to significant consequences of a regulated or normalized rhythm.[7]

Animal studies enable researchers to examine how one might dysregulate the cortisol rhythm with a chronic stress load. In one study, a mild stressor was repeated for 1, 3, and 5 weeks. As the chronic stress load was increased, the normal low level of corticosterone that would be expected at the beginning of the inactive period increased. The chronic stressor increased the diurnal trough corticosterone levels and flattened the circadian corticosterone rhythm.[2]

The different parameters of CFS suggest possible causes, including infection causing a chronic subthreshold immune activation, physiological dysregulation, endocrine dysregulation, circadian dysregulation, or sleep dysregulation. However, it might be difficult to differentiate between cause and consequence, and results of any of these dysregulations can contribute to other dysregulations and feed into CFS. Several mediators may be involved—activated immune cells, cytokines, hormones, neurotransmitters, or other yet-unknown factors.

Stress can have important effects on any of these activities, and it can also have an effect through the described mechanisms. There are many ways in which stress, either intrinsic or extrinsic, might affect the progress or the nature of CFS. The fact of having CFS itself might affect a patient's stress load. The interrelated nature of issues associated with CFS emphasizes the need for multidimensional, multifactorial approaches, and that hypothesis-driven studies might also be useful in understanding relationships of the factors and determining cause versus effect.

Discussion

Dr. Buchwald: Many aspects of CFS invoke an explanation using your chronic stress model, and others are more reminiscent of your acute stress response. For example, many CFS patients report that they have been under chronic stress just prior to the onset of illness. A number of individuals also report various kinds of victimization and trauma early in childhood or in their adult life, and most people who are studied are chronically ill at the time of the study. There are also various more acute aspects to the illness—for example, the acute onset, the impaired response to acute stressors (by self-report), and exacerbations of the illness itself. How would you parse out those effects in studies of CFS or, alternatively, what might you expect to see in studies on stress and augmentation of the immune response?

Dr. Dhabhar: In general, it seems that the acute stress system is tapped to enhance the immune response that is commanded by the antigen-presenting situation at the time the stressor is experienced. Some responses

benefit from or are enhanced by having an IL-2 interferon gamma response, and the acute stressor can enhance them. In diseases like CFS and many pro-inflammatory disorders, the IL-4/IL-10 driven immune responses may be the problem. One might ask whether in CFS acute stress drives up IL-4/IL-10 and, if so, how? One might also ask at what point is the ability to mount these responses lost, relative to an increasing and immunosuppressive chronic stress load? In a study that explains the phenomenon of burnout in shift-working nurses, it was found that, after a point and because of the exceeded chronic stress load, there is no acute stress response. Perhaps CFS is a case of the acute stress response gradually being lost in favor of a more chronic stress load. Other evidence is that the chronic stress load seems to push the IL-4/IL-10 response, which has a reputation of producing more immunopathological reactions.

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Evaluating Immune Function in CFS

Nancy Klimas, M.D.

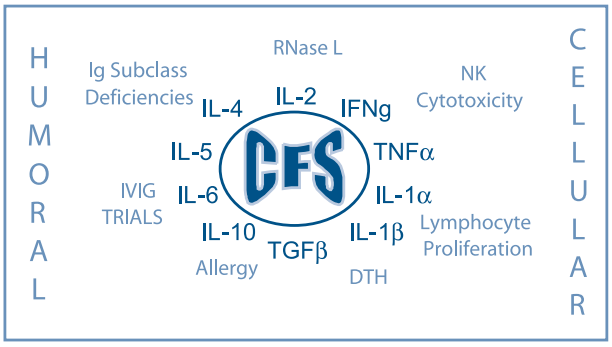
One of the most significant problems in evaluating immune function in CFS occurs because all CFS patients are often grouped together under one large umbrella when, in fact, the population is heterogeneous. Subpopulations must be defined, but investigators have not agreed on those definitions. To date, the problem has been either ignored or individual researchers have defined subpopulations for the purposes of their studies. If cases are classified by underlying dysfunctional systems, broadly overlapping circles of immune, autonomic, and HPA axis dysregulation are seen, but researchers do not know the extent of overlap. Scientists lack the tools, the consensus, and the empirical data needed to create the definitions that would allow research to move forward more quickly and with consistency.

Hundreds of papers have explored immune dysfunction in CFS, with much attention to cytokine dysregulation. Type 1 or type 2 cytokine patterns are often referred to as IL-4/IL-10, the pattern that would be predicted in a chronic stress model. The literature is replete with papers that describe type 2 dysfunctions and pro-inflammatory cytokine expression in at least some subpopulations of CFS. Considering the meaning of these patterns in the context of cytokine dysregulation, type 1 cytokines enhance natural killer cell function. The natural killer cell function has been described as subnormal in CFS patients, so this is a plausible conclusion.

Subpopulations must be defined, but investigators have not agreed on those definitions.

Type 1 cytokines are needed for T cells to interact appropriately and grow in culture, and lymphocyte proliferation data suggest that this does not function as it should in some people with CFS. Type 2 dysfunction tends to be associated with humoral factors, specifically immunoglobulin E (IgE) production, which creates allergies and sets up subsequent antibody-mediated dysfunction and autoimmune consequences.

A number of research issues became evident in a review colleagues and I conducted of more than 100 papers on immune abnormalities published in the past 2 decades by 44 different research groups. Case definitions have changed over time although since 1994 there has been widespread use of the international case definition of 1994 (the Fukuda criteria). We weighted our assessments by the number of relevant papers and the consistency of findings.



In reviewing immune activation data, it is necessary to view the whole body as a three-dimensional or over-time description of the process of immune activation. For example, when considering the RNA cell interferon induction pathway, the focus is on intercellular events that will eventually result in some cell membrane expression downstream that enhances the cell function, and on peptides produced by the cell that would circulate in the serum. However, comparisons have been difficult because researchers have used a number of different methods to try to define the state of immune activation. Other evidence of immune activation finds that naive cells tend to be present in larger numbers in a less activated patient, and once activated they become memory cells. Memory cell population in higher

numbers in people with CFS has been shown by four groups (413 patients in one set of studies and 71 in another), with some dissenting studies.

	# Studies (Total Subjects)	
	Abnormal	Normal
+ CD5+19+	3 (312)	
+ RNase L	3 (135)	
+ CD26	1 (30)	
+ Activation	18 (2734)	2 (248)
- NKCC	7 (164)	1 (26)
- CD45RA	3 (413)	1 (71)
+ CD28	2 (162)	1 (101)
+ CIC's	3 (1018)	2 (97)
- IgG 1&3	3 (553)	2 (41)
+ ANA	4 (1110)	4 (96)
- LPA	4 (523)	4 (78)
+ Neopterin	3 (191)	3 (130)
- DTH	1 (100)	1 (26)
+ Allergy	3 (353)	4 (143)
+ CD38	3 (309)	4 (125)
+ TNFα	4 (334)	6 (110)

Immune activation can be studied with several different markers. Literature covering approximately 3,000 patients supports the finding that immune activation markers have been found in higher numbers in CFS patients than in control populations. Some of those markers include:

- CD38 is a specific marker for a particular type of cellular activation. It has been described by a number of groups.
- CD28, using a marker on the suppressor T cell, has been the subject of fewer studies. Natelson's research found CD28 in one group of subjects and not in others, illustrating that different results are seen even within a single research site.
- CD26 is a marker that may be associated with symptom severity. An ongoing study of the use of cognitive behavioral therapy (CBT) looks at immune markers as they relate to markers for severity of illness; results indicate that the immune markers, particularly CD26, correlate with flu-like and fatigue symptoms.[1] The correlations are strengthening as the study continues. Two CBT studies of two different CFS patient populations have confirmed the association of CD26 with illness severity, and when that is weighed with low natural killer cell function, the association is even stronger.[2,3]
- Neopterin is inexpensive, in serum (therefore easy to use), and without the need for flow cytometry. Although not as sensitive a marker as the flow markers and not as reliable, it still has some usefulness in clinical studies. In one study, high neopterin was not found in a CFS population until a severity of illness split was done. After that split, investigators found high levels of neopterin in CFS patients with high cognitive difficulties, but not in people without cognitive complaints. This finding underscores the importance of defining and studying CFS population subgroups.

Laboratory Evidence of Immune Activation

Supporting Studies

Cheney, '90: IL-2
Klimas, '90, '94, & '97 CD45RA/ HLADR
Chao, 1990: Neopterin / IL-6
van Greure, '90: HLADR
Landay, '91: HLADR / CD38
Gupta, '91 & '97: CD11a/IL-6/ TNFα
Tirelli, '93 & '94: HLADR/ CD38/CD11b
Hilgers, '94:TNFα /ANA /IC's
Matsuda, '94: Neopterin
Suhadolnik, 94: Rnase L
Natelson, '95 & '01: C3 / Rnase L / IL-4
Bates, '95: Immune complexes
Conti, '96 Eosinophil Cationic Protein
Buchwald, '97: B2M / Neopterin
Bennett, '97 & 99: TGFβ / IL1β / IL-6 / a2M
Mawle, '97: IL-2 / IL-6 /TGFβ
Hassan, '98: HLADR
Borish, '98: IFNα / TNFα
Moss, '99: IFNα / TNFα

Dissenting Studies

Straus, '93 HLADR / CD38: frozen cells
Swanink, '96: HLADR / CD38 / TNFα
Visser, '97: CD45RA: frozen cells
Peakman, '97: HLADR/CD38: Frozen cells
(Natelson, '98 & '99: HLADR / CD38)

Cytokines are a problematic measure. They are descriptive, providing a helpful sense of what is occurring, but difficult to measure reliably. Messenger RNA upregulated for cytokines is a sensitive marker being used by a number of researchers. Another widely accepted method of measuring cytokines is to put lymphocytes into a culture and stimulate them, and then measure how much cytokine the lymphocytes make in the maintenance of that culture. Some published data support using combinations of these markers or methods to sort out the cytokine problem. TNF-α is one of the more interesting cytokines. Cluster analyses have shown the pro-inflammatory cytokine cluster in the type 2 cytokine pattern, plus around TNF-α, which seemed to be a central core cytokine in expression. Other research has found that cross-sectional work was not useful when looking at cytokines, because expression varies according to symptom severity, with the most severely affected patients expressing cytokines most significantly. Following patients longitudinally reveals a changing pattern, limiting cross-sectional study and highlighting the need for care in designing models. Longitudinal design should be a key component of any model study because the effects of duration and severity of illness at any given point can significantly affect markers.

In one study, CFS patients were shifted from type 2 to type 1 cytokine expression. This was an elaborate study in which researchers extracted lymph nodes, grew cells and forced them *in vitro* into type 1 patterns, then reinfused them into patients, thus forcing the type 1 cytokine pattern upon a patient. In six of the seven patients in whom this change was achieved, significant clinical improvement occurred in cognition, fatigue, and overall quality of life. This was an important observation, although it could be deemed an indirect consequence. A placebo-controlled trial is needed for the result to be considered reliable.

Studies of immune complexes circulating in antinuclear antibodies in CFS patients are also interesting but none have yet been conducted that take into account duration of illness. Whether long-term patients are making measurable autoantibodies or whether autoantibodies are mediating the illness in early onset patients is not known. This type of model suggests that duration should have an effect and that longer-term patients would be more likely to express measurable autoantibodies.

A similar question considers whether allergy is a consequence or a cause. While there is general agreement that the cause of CFS is poorly understood, data suggest that some CFS patients have measurable increased allergy expression; there are also some dissenting studies.

Natural killer (NK) cell data are more consistent and a large number of groups have described NK cell dysfunction in CFS populations; however, some researchers have failed to find that effect. This dichotomy points to the need for subgrouping—NK cell dysfunction is a characteristic of one subgroup of patients who might be more likely than others to respond to immunomodulatory approaches. Ongoing work on the mechanisms of NK cell dysfunction is examining perforin content. CFS patients have been found to have abnormally low levels of perforin in NK cells compared to healthy controls, but similar levels to patients with human immunodeficiency virus (HIV). Perforin may be a useful surrogate for NK cell function because it can be frozen and thawed, whereas NK cells are not reliable after thawing. Different methodological strategies are being examined to improve reliability across research centers.

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Survivors of Hurricane Andrew, which devastated parts of south Florida in 1992, constitute the cohorts for a number of studies. Initially the hurricane was considered an acute stressor, but continuing hardships changed the storm into a chronic stress model. Patients with low cognitive difficulties had much better immune function (measured by phytohemagglutinin T cell function *in vitro*), again indicating that cognitive difficulty seems to be a useful split line on cell function. In a CFS population, patients with the lowest NK cell function had the most severe fatigue and the most severe cognitive difficulty, as measured by passive digit span tests and cognitive difficulty scores. However, patients with normal NK cell function had more depression. Depression did not seem to be a mediator of the cognitive findings or severity of illness.[4]

T and B cell function *in vitro* were also measured; these tests are tedious and expensive and not often done, but the predominant literature suggests abnormalities in T and B cell function. Only a few studies have been conducted on delayed-type hypersensitivity (DTH) in CFS populations, but Lloyd's group has shown that DTH is reduced in CFS patients. In an ongoing prospective study of acute infectious illness by that group, it appears that reduced DTH predicts long-term fatiguing illness after acute viral or bacterial illness.

In the review of the body of work in this field, a number of limitations became apparent. A problem throughout the literature is population size, with most studies using low numbers that result in problematic statistical power. Problems are also apparent in describing study populations and specifics of study design. In all such studies, researchers need to thoroughly explain their methodologies.

Since CFS is primarily a disease of women, time of hormonal cycles when blood samples are drawn may be relevant and should be recorded. Circadian rhythms are also relevant to immune function, particularly in the CFS population. Longitudinal

relapse pattern is another factor that has not been taken into account in many studies, and using longitudinal designs is critically important. Cross-sectional studies are limited; they do not, for example, indicate whether immune activation is a preceding or a coincidental event, follows the periods of exacerbation, relates to symptoms, or explains the nature of the autonomic and/or endocrine mediators.

Concerns about laboratory methodology are mentioned only rarely in the literature. Many samples are volatile and must be frozen quickly to yield a reliable measure. The reference laboratories must be competent. Some measures are not reliable after shipping by typical overnight services because the samples must be kept at certain temperatures.

Consistent measures and a consistent panel for measuring a highly volatile and interchangeable system should be established; a five-point panel may not be appropriate because there are not five measures that adequately describe what is occurring in CFS. A common protocol and commonalities in goals for CFS researchers are needed. Reference populations vary and should be reviewed—while study populations are usually women, often the normal controls are not gender matched. People skilled in study design and analysis should become involved in setting future directions for CFS research. Multicentered longitudinal studies with well-described populations and methodologies are needed. Before studies are implemented, method and expertise of research groups should be considered, so that groups most suited to a specific project can become involved. Proficiency standards must be established.

Discussion

Dr. Van Konynenberg: It is well supported that most people with CFS have a history of some sort of stress, and sometimes a combination of stressors over a long duration, and it is well supported that the body responds to stress with the HPA axis. Assuming that people with CFS are healthy until they become ill, particularly acute onset patients, we can also assume that the HPA axis was working normally until they became ill. That means cortisol and epinephrine were elevated, sometimes over long periods of time. Suppression of the immune system occurs, resulting in the onset of conditions that TH-1 is supposed to defend against, but TH-1 is not there to do so. At this point in the pathogenesis, the depletion of glutathione comes into play. At the last meeting of the American Association for Chronic Fatigue Syndrome, a report on a study showed glutathione depletion. Why is that important and how does it happen? We know that epinephrine is elevated. We know that a certain fraction of epinephrine oxidizes to form adrenochrome, one of the oquinones that the body deals with using glutathione. The Phase II detoxification for the oquinones is glutathione conjugation, so there is a mechanism to deplete glutathione from elevated epinephrine secretion over long times. Other things also serve to deplete glutathione as well, including toxin. So glutathione decreases and that also suppresses TH-1, and then comes the onset of the viral infection.

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Stress and Antidepressant Modulation of the Limbic HPA Axis

Juan F. López, M.D.

Chronic fatigue syndrome and depression are comparable in many ways. It is important for people who study CFS to know about depression and for people who study depression to know about CFS. Traditional phenomenological categories may no longer be relevant and the field may be dividing itself artificially. A great deal can be learned about diseases by comparing their common aspects, such as common symptoms.

The HPA axis, the classic neuroendocrine system that responds to stress, is not the only system that responds to stress. The system is geared to making glucocorticoids (cortisol in humans and corticosterone in rats). Both the peripheral organs and the brain are targets for glucocorticoids. The HPA axis can be referred to as the limbic-hypothalamic-pituitary-adrenal axis, because limbic components are an integral part of the system, particularly the hippocampus, which is an important area for memory and mood and a target for HPA activity.

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The stress response is an adaptive response that, under conditions of chronic demand, becomes maladaptive. HPA axis dysregulation exists in multiple diseases, including depression, hyperactivity, and CFS. The paraventricular nucleus (PVN) of the hypothalamus, where corticotropin-releasing hormone (CRH) is produced, lies at the junction of multiple circuits and neurotransmitters, with many different inputs. Regarding the acute effects of antidepressants, the monoamines and adrenaline and serotonin have most of the input to the PVN. In general, monoamine release activates the system. An acute injection of monoamines in or peripheral to the hypothalamus will trigger an acute release of CRH, ACTH (adrenocorticotropin), and cortisol. As a mechanism of antidepressants, particularly tricyclic antidepressants and serotonin reuptake inhibitors, a monoamine neuron and norepinephrine are released into the synapse and then taken back by a transporter. Antidepressants block the uptake area, in effect allowing more monoamines in the synapse.

This acute effect is necessary for antidepressant action, but is not the only necessary step because antidepressants to treat mood disorders and other diseases do not work immediately; days, weeks, and sometimes antidepressant action is observed only after months. Current thinking is that chronic modulation of some of the receptors is what underlies the antidepressant action. Blocking reuptake acutely results in an increase in norepinephrine, and an acute antidepressant administration also causes an increase in ACTH.

Animal Studies

My laboratory examined the effect of antidepressants under baseline conditions. In one study, rodents were injected with imipramine, a tricyclic antidepressant, and corticosterone was measured 24 hours after each injection. After 3 days of injections, an enhancement of imipramine was seen in the synapse as was an increase in corticosterone, although the findings were somewhat obscured by "noise."

The HPA axis is geared to maintain optimal cortisol levels and is difficult to dysregulate because this is such an important function. The system seems normal before people become ill, but it may be working hard to compensate to keep the cortisol level normal. This compensation may be what ultimately causes the dysregulation. Researchers are beginning to look at the effects of antidepressants and stress in the brain with the idea that, even though the cortisol

levels are normal, dysregulation is occurring at other sites such as the limbic brain and particularly the hippocampus.

Two types of corticoid receptors exist in the brain: the mineralocorticoid receptor (MR) and glucocorticoid receptor (GR). They mediate the action of glucocorticoids in the brain and periphery. MR has a more limited distribution in the hippocampus and septal regions than GR; GR has a wider distribution in the hippocampus, hypothalamus, and frontal cortex. MR and GR are also important because they control the activity of the HPA axis: they signal the brain to shut the system down under normal circumstances. Classically, MR is high-affinity, low-capacity—it avidly takes on glucocorticoid but saturates easily—whereas GR is low-affinity, high-capacity.

With exposure to a stressor, MR becomes fully occupied, and GR takes over and then shuts the system down. Some scientists believe that MR is more important for circadian rhythm. MR and GR are important for both acute and chronic stress because they are both occupied when the organism is stressed. It has been proposed that an MR/GR balance is essential for maintaining the tone of the HPA axis; it is also essential for the homeostasis of the organism and how the organism responds to stress.

An altered MR/GR balance is a possibility in many of the diseases associated with stress, as noted by a number of researchers. We use a model of chronic unpredictable stress (CUS) to study the GR antimargin expression and the effect of antidepressants. In our study, rodents were stressed with different stressors daily at different times of the day, so that the time and nature of the stressor was unpredictable. (Stressors included a swim, restraint, cold, noise, ether, and crowding.) Unpredictability adds psychological stress to the physical stress. Rodents have been found to be resilient to more predictable stressors, which cease being a stressor as the animal becomes accustomed to it.

Some behavioral abnormalities are seen in this so-called animal model of depression (which is not a useful label because it is not possible to determine whether a rat is experiencing mood problems). Animals can help focus researchers on specific symptoms or behaviors that may be amenable to physiological studies, and research has shown that behavioral "abnormalities" are reversed by antidepressants. CUS produces HPA hyperactivity, and similar receptor changes have been seen in postmortem tissue examinations of humans with major depressive disorder.

In one study, rodents exposed to chronic unpredictable stress were treated with either desipramine, a tricyclic antidepressant that is also a potent norepinephrine uptake inhibitor; or with zimelidine, a selective serotonin reuptake inhibitor (SSRI). The corticosterone levels were increased a small amount by both drugs, and chronic stress caused an increase in baseline corticosterone. When desipramine was administered to the stressed animals, it blocked the stress-induced corticosterone increase, but zimelidine did not have a similar blocking effect.

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To understand the mechanisms at work, we examined MR and GR levels in the hippocampi of the sacrificed rats. CUS was seen to cause a downregulation of MR in the hippocampus, and desipramine blocked downregulation but zimelidine did not. The antidepressant that was unable to block the stress response was also unable to block the downregulation. Sometimes downregulation is seen with GR and sometimes it is not, depending on the nature of the stressor and the pressure and strain on the rat. It can be difficult to decrease and easier to increase gene expression when the transcription rate of a gene is already low. Some researchers have reported seeing downregulation of GR in the hippocampus.

The data suggest a link between peripheral corticosterone response or lack of it and MR regulation, but it is unknown whether MR downregulation is seen with chronic stress mediated by peripheral cortisol and how that can be tested. We performed adrenalectomies on another group of stressed animals, hypothesizing that, if the adrenal is removed, the corticosterone response would be gone because the glucocorticoids would be absent. If it is a central response issue, the response should still be seen. One group of animals were adrenalectomized and another adrenalectomized group was given cortisol

in their drinking water, which helped maintain the circadian rhythm but eliminated the stress response. We also looked for GR in the prefrontal cortex. The stressed animals showed an increase in baseline corticosterone but within normal limits, but downregulation was seen in some of the subfields with chronic stress. In general, eliminating the corticosterone response eliminated the MR downregulation usually seen with chronic unpredictable stress. The GR also decreased in the stressed animals.

Another study tested and compared fluoxetine with several different SSRIs. The animals were subjected to 28 days of chronic unpredictable stress. The control group in the study was an unhandled group, and a mildly stressed group was injected with saline. Corticosterone increased slightly in the saline group. Fluoxetine alone did not have an effect, but fluoxetine plus stress resulted in a significant increase in corticosterone, a dramatic and unexpected finding.

Antidepressants clearly buffer the effects of stress, which may have clinical applications to CFS.

One conclusion to be drawn is that antidepressants have a different effect on a baseline organism than on an organism under stress. Fluoxetine blocked the increase of corticosterone in the group injected with saline (the mild stressor) but it did not block it in the CUS group; fluoxetine does not block the stress response of a severe stressor that might be involved with the epinephrine system. In the brain, there was no difference in MR between the saline group and the unhandled group. However, even though fluoxetine did not block the corticosterone response, it did seem to prevent the amygdala regulation: it seemed to protect the hippocampus from the effects of corticosterone, even though it did not block the corticosterone itself. This result was not observed in the frontal cortex. Downregulation of GR was seen in the frontal cortex and did not appear to be prevented by fluoxetine.

Researchers are still trying to establish the meaning of these findings. Differential circuit effects have been observed, an important consideration with antidepressants. Different antidepressants might have different effects on the HPA axis, depending on what part of the axis is examined. Chronic stress can modulate glucocorticoid receptors, and this modulation, at least the downregulation, seems to be mediated by a group of corticoids.

Antidepressants clearly buffer the effects of stress, which may have clinical applications to CFS. Failure of an antidepressant to block or modify the stress response is associated with failure of that antidepressant to correct the corticosteroid receptor changes. In this particular animal model, tricyclic antidepressants may be more effective than SSRIs in preventing these stress-induced corticosteroid receptor changes.

Human Studies

Downregulation of the mineralcorticoid receptor is seen in unmedicated patients with a history of depression and suicide. Even though MR is only found in the hippocampus in rodents, it is found in the cortex of humans and probably plays a wider role in humans than in rodents. This is a reminder that some animal findings apply to humans and some do not.

Serotonin is implicated in multiple functions, including mood, pain perception, sleep, and appetite. Part of the reason these multiple functions are possible is that serotonin projects to many different brain areas, such as the thalamus, the hippocampus, and the amygdala. There are multiple serotonin receptors; by 1993, 14 serotonin receptors were known, which gives serotonin many ways to modulate multiple functions. Most aspects of depression seem to be related to the 2A and 2C receptors; both are altered in suicide and depression, and both are known to change with antidepressant treatment.

In the human hippocampus, the glucorticoid receptor is found on MR receptors, indicating that they must “talk” to each other. Researchers have seen downregulation of MR in the hippocampi of patients with mood disorders in postmortem studies. Several studies show downregulation in multiple areas of the brain. Other findings include decreased receptor number and mRNA levels of the serotonin 1A receptor subtype (5-HT1a) in the rat hippocampus and prevention of the 5-HT receptor changes with antidepressants. The serotonin 2A receptor has been shown to be increased in the prefrontal cortex of suicide victims suffering severe depression, and many antidepressants downregulate serotonin receptor binding.

Conclusions

Serotonin and the HPA axis are related in a variety of ways. Serotonin can increase release of CRH but, more importantly, serotonin receptors are co-localized with glucocorticoid receptors in the brain, especially in the hippocampus. Many researchers have shown that glucocorticoids can modulate serotonin receptors. In the case of adrenalectomy, significant regulation of 1A in the hippocampus is seen, but some resupply of corticosterone normalizes this regulation. The receptor is more sensitive to glucocorticoids. What happens when an animal is chronically stressed? Antidepressants have different actions in stressed and nonstressed organisms. An example is what happens with the 1A receptor—there is a downregulation of 1A with chronic stress, but antidepressants prevent this downregulation. Stress also causes change in the serotonin 2A receptor: the glucocorticoids upregulate 2A receptors (important for mood and sleep) in the same direction as seen in depression. Antidepressants that block the corticosterone response prevent that upregulation. But fluoxetine or zimelidine (the SSRIs) do not block the corticosterone response or the 2A upregulation. Failure to block the stress response is associated with a failure to correct the 5-HT receptor changes. In the animal models studied, tricyclic antidepressants were more effective than SSRIs in preventing stress-induced 5-HT receptor changes, at least with severe chronic stress.

Applications to CFS

Our research found GR and MR dysregulation in postmortem tissue of patients with mood disorders and wondered if the same downregulation would be seen in CFS patients. Researchers should conceptualize CFS as a brain disease and correlate clinical observations with brain studies, including *in vivo* PET scanning and brain collections as used in the study of mood disorders. Brain collections provide an opportunity to examine mRNA and conduct detailed cellular studies.

It is clear that the HPA axis dysregulation itself can cause or worsen serotonin receptor abnormalities in some CFS patients. Glucocorticoids can directly affect serotonin receptor levels, which suggests a means to regulate the receptors in the case of serotonin function problems. Glucocorticoids and many other hormones (for example, estrogen) modulate serotonin receptors. Correcting the HPA axis dysregulation may be necessary for a treatment response to occur; patients with uncorrected HPA are more likely to relapse after treatment with antidepressants.

Regarding the differential effects of medication in the HPA axis, the goal in a hypocortisolemic state is to increase cortisol levels; one suggested technique is to use positive stress such as exercise. Some data suggest that people with atypical depression have low cortisol levels, and SSRIs are more effective for atypical depression than are tricyclic antidepressants. There is an optimal level of glucocorticoids for good brain function: too much or not enough can have important implications for how many systems work.

In one study, patients with CFS receiving low-dose dexamethasone (synthetic cortisol) showed hypersensitivity to feedback.[1] This raises the question of whether the brain will perceive the enhanced sensitivity as increased cortisol, even though the levels are low. A differential sensitivity may be important; multiple systems are involved with CFS, and it is necessary to understand how they interact.

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Discussion

Dr. Van Konynenberg: A paper by Logan 2 years ago indicated that in CFS, serotonergic activity is elevated, and a recent paper by the Glasgow Group showed high tryptophan and low tyrosine in the blood of CFS patients. Tryptophan is the precursor of serotonin and tyrosine is the precursor of dopamine, so they are arguing that serotonergic activity is high and dopaminergic activity is low.

Dr. Ottenweller: In our work at New Jersey Medical School, researchers have been trying to connect stress and CFS. Your stress models of the animals show hypocortisolemia, predicting low levels of cortisol for humans. We do not see low levels of corticosterone in any animal stress model and our group has worked with half a dozen.

Dr. López: Not all stress works equally, which is why we went to chronic unpredictable stress. We do not always see big changes. It has to do with the nature of the stressor. Also, some people adapt to stress. As I said earlier, the system is tightly regulated to maintain normal levels. Sometimes it must be pushed to see differences.

Some of the rodent work dealing with antidepressants and early life stress might have some applicability to CFS. When a pup is stressed and with the mother, there is little corticosterone response; but when the mother is removed, there is a large response. When pups were injected with desipramine, the response matured so that five of the animals not responding to corticosterone responded, even with their mother. Sometimes antidepressants may be effective in bringing a system back to normal.

Stressors are different; stress is not depression and stress is not chronic fatigue. These things are predisposing. If the body has a weak link, a stressor will take advantage of it. Early life stressors have been implicated in medical illnesses, depression, and CFS, almost at a comparable level to genetic vulnerability.

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HPA Axis and Autonomic Nervous System Function in Fibromyalgia

Gail Kurr Adler, M.D., Ph.D.

An internal biological clock has an important influence on the 24-hour biological rhythms for hormone levels (adrenocorticotropic hormone [ACTH], cortisol, melatonin, and growth hormone), sleepiness and wakefulness, subjective mood, objective performance, and core body temperature. Misalignment of the endogenous circadian rhythm with the sleep/wake cycle causes disturbed sleep at the desired sleep time, fatigue and problems in remaining awake during the day, impaired cognitive function, gastrointestinal upset, and inappropriate timing of hormone release. Misalignment is sometimes seen in individuals experiencing jet lag or engaging in shift work, and in some people who are blind.

A procedure called the "constant routine" can be used in humans to help identify internal circadian rhythms. The constant routine consists of 40 hours of uninterrupted wakefulness, with constant posture, hourly small meals, controlled activity, and low light levels to measure melatonin, which is suppressed by light. The goal of the constant routine is to carefully control the environment in order to uncover an endogenous biological rhythm. For example, during a normal 24-hour day with a normal sleep/wake cycle, a person's core body temperature dips during sleep; however, it is not clear whether that dip is related to sleep or to an endogenous circadian rhythm. In the constant routine, a dip in core body temperature is seen during the habitual sleep period indicating a circadian rhythm in core body temperature. Sleep intensifies the dip in core body temperature, showing that both sleep and the internal biological clock influence core body temperature.

The symptoms associated with circadian misalignment occur in patients with fibromyalgia and chronic fatigue syndrome, raising the question of whether circadian rhythm misalignment could be occurring in patients with these conditions. Dr. Adler and colleagues designed a study protocol to address this question in fibromyalgia patients.[1] The subjects were premenopausal women who were not taking any medications (including any type of steroid, even if applied topically or taken only transiently) during the past year and who were not depressed. The no-steroid requirement eliminated almost 30 percent of the group's fibromyalgia patients and 10 to 20 percent of the healthy controls. All subjects were studied in the follicular phase of the menstrual cycle. For 3 weeks before the study, to establish baselines, the subjects kept a sleep/wake log and followed their normal sleep/wake cycle, which they were asked to keep regular. A total of 15 fibromyalgia patients and 14 controls were studied.

After the 3-week period, the subjects were admitted to the hospital and their sleep was monitored 3 nights in a row. The first night was considered a night of acclimation, when subjects often do not sleep well. The second night was a night of recovery sleep. The third night's sleep, which was lying flat in the dark, was considered a better reflection of normal sleep. The constant routine began when the subjects awakened after the third night. The head of the bed was raised to 30 degrees and the lights were adjusted to a very low level, 10 lux. Every 20 minutes the subjects filled out a computerized questionnaire asking if they felt alert or sleepy. A normal circadian rhythm in sleepiness and wakefulness was seen in both control and fibromyalgia subjects. Stiffness and pain were also measured and no association with circadian rhythm was noted, although fibromyalgia patients complained of more stiffness and pain than did the controls. Analysis of melatonin, cortisol, and core body temperature found no differences between the subjects and controls. Thus, individuals with fibromyalgia show no evidence of circadian rhythm misalignment. Furthermore, cortisol and melatonin levels did not differ between the control and fibromyalgia subjects.

In a subset of control and fibromyalgia subjects, heart rate was studied during the sleep period and the following 40-hour constant routine. While the controls had the expected slower heart rate during the sleep period, the fibromyalgia patients did not. Examining high frequency and low frequency as a percentage of total power through spectroanalysis of heart rate revealed some shifts between sleep time and constant routine. For each of these parameters, mean values were calculated for the 8-hour sleep time and the 8-hour habitual sleep time during the constant routine. The results showed differences in autonomic control of heart rate between the two time periods in the different subject groups. However, no differences were found in total sleep time, or amount of time spent in rapid-eye-movement sleep or stages 1, 2, 3, or 4 of sleep. These findings suggest significant differences in heart rate and heart rate variability during sleep between subjects with fibromyalgia and healthy controls, differences not due to sleep architecture or alterations in circadian phase. Patients with fibromyalgia may have decreased parasympathetic and increased sympathetic nervous system modulation of the sinus node compared to healthy women.

In another study, autonomic function was examined with the application of the stimulus of low blood sugar.[2] This was done using a hypoglycemic, hyperinsulinemic clamp and a constant insulin infusion, with serum glucose measured every 5 minutes. The rate of glucose infusion was adjusted to maintain the patient's glucose at a set level. Glucose was kept at a normal level (90 mg/dl) for 30 minutes, then dropped every 30 minutes, down to 40 mg/dl, providing a precise, quantifiable, exogenously controlled hypoglycemic condition that was not affected by the subject's endogenous hormone release. Hypoglycemia stimulated a rise in plasma epinephrine in healthy controls. This increase in epinephrine was approximately 30 percent lower in the fibromyalgia patients. In a provocative related finding, the Fibromyalgia Impact Questionnaire was used to obtain an overall assessment of activities of daily living, and patients with the worst health status had the lowest epinephrine response to hypoglycemia.

Hypoglycemia is also known to turn on the HPA axis, and this response was studied in the same group of patients. When patients are treated with cortisol, it feeds back and turns off CRH and ACTH; when treatment ceases, the HPA axis is suppressed and patients may experience symptoms of steroid withdrawal, including fatigue, malaise, muscle aches, sleep disturbances, gastrointestinal complaints, difficulty thinking, dizziness upon standing, fever, and nausea—all complaints seen in CFS and fibromyalgia patients. Researchers in this study were looking for HPA axis disturbances in fibromyalgia patients. ACTH rose in response to hypoglycemia in fibromyalgia patients, but to a lower level than in controls. Similar prolactin responses to hypoglycemia were observed in control and fibromyalgia subjects. Possible explanations for the reduced ACTH response could be a defect in signals coming from the hypothalamus or a defect at the level of the pituitary. Three of four published studies report a significantly increased ACTH response to an infusion of the hypothalamic secretagogue CRH, while one reports a normal-to-increased response.[3-6] These studies argue against a primary defect at the level of the pituitary and may indicate a problem in hypothalamic function. The elevated ACTH response could result from chronically low CRH levels, leading to upregulation of CRH receptors on the pituitary and producing an exaggerated response when CRH is administered.

These findings suggest significant differences in heart rate and heart rate variability during sleep between subjects with fibromyalgia and healthy controls, differences not due to sleep architecture or alterations in circadian phase.

The cortisol responses to hypoglycemia were similar in control and fibromyalgia subjects. In addition, ACTH dose response curves performed with infused ACTH showed a normal cortisol response in fibromyalgia patients. Infusions with angiotensin II in a dose response fashion in patients with a low salt balance also showed a normal aldosterone response. Thus, the adrenal responds normally to both cortisol and aldosterone secretagogues.

In seeking an explanation of the changes in ACTH and epinephrine seen in the above studies, researchers looked for a way to link the HPA axis and autonomic nervous system. A body of data from animal and human studies suggests important interactions between the two systems. A series of studies performed by Davis and colleagues have examined the effect of an antecedent stress on the autonomic and hormonal response to subsequent hypoglycemia in healthy humans.[7] The subjects underwent a series

of 2-day studies separated in time. On day 1, the subjects received either a stress or a placebo stress and on day 2 the hormonal and autonomic nervous system responses to hypoglycemia were assessed. Subjects who, on day 1, experienced prolonged hypoglycemia or very vigorous exercise showed reduced hormonal and autonomic responses to the day 2 hypoglycemic stress; specifically, reductions in the ACTH, autonomic nervous system, growth hormone, beta-endorphin, and pancreatic polypeptide responses to hypoglycemia. Day 1 hypoglycemia also reduced the hormonal and autonomic responses to day 2 exercise, indicating that antecedent stress can blunt the responses to a variety of stressors. Subjects who, on day 1, received an infusion of cortisol designed to raise circulating cortisol levels to those observed during stress also had markedly reduced hormonal and autonomic response to day 2 hypoglycemia. One resulting hypothesis is that stress-induced rises in cortisol, through inhibition of CRH, inhibits the ACTH, hormonal, and autonomic responses to subsequent stress.

These results suggest a link between the HPA axis and the autonomic nervous system in their responses to stressors. This finding has important implications for hypoglycemia-associated autonomic failure, a syndrome of hypoglycemic unawareness in insulin-dependent diabetes patients who have frequent hypoglycemic episodes and lose their ability to perceive the symptoms of hypoglycemia, without the usual response of increased epinephrine or cortisol. In these patients profound and prolonged hypoglycemia can lead to coma and death. When blood glucose is controlled and the stress of hypoglycemia is carefully avoided, the hormonal and autonomic nervous system responses improve over several weeks.

A working model for the pathophysiology of fibromyalgia should include genetic factors, gender effects, environmental factors, and use of steroids or other drugs, all of which can be implicated in the decrease in CRH or autonomic function (see Figure 1). Because of the feedback between these two systems, a defect in one may lead to a defect in another. Many animal studies suggest that both the autonomic nervous system and CRH can influence pain perception, which may explain the increased sensitivity to pain in fibromyalgia patients. Both of these systems influence sleep, gastrointestinal function, cognitive function, blood pressure, and hormone levels.

In fibromyalgia patients, a therapeutic goal might be to increase the dynamic range of the normal rhythms in HPA axis and autonomic function, so that when a stress occurs, the systems are turned on and are also capable of being turned off appropriately. If these two systems are suppressed, attention should be given to ways in which their activities can be increased.

These results suggest a link between the HPA axis and the autonomic nervous system in their responses to stressors.

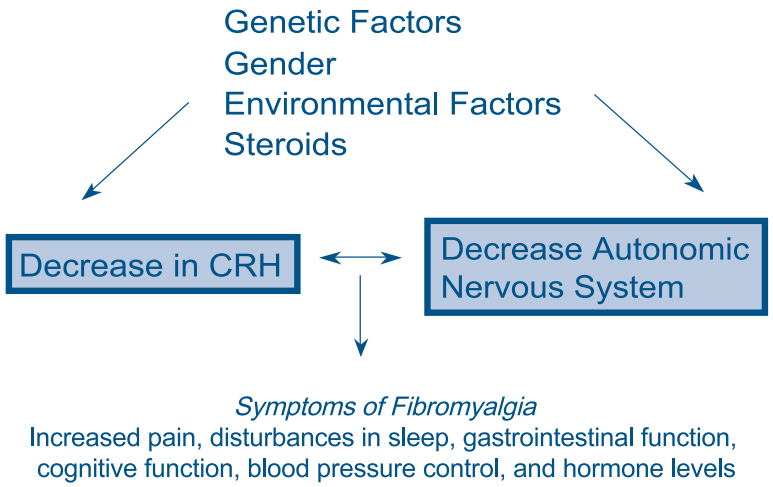

Figure 1. A working model for the pathophysiology of fibromyalgia.

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Session Two: The Autonomic Nervous System

Chair: David Goldstein, M.D., Ph.D.

A major theme of chronic fatigue syndrome research (and psychosomatic research in general) is that stress regulates the inner world by way of the autonomic nervous system. The brain interacts with the external environment—the outer world—by way of the central nervous system and its effects on skeletal muscle. The autonomic nervous system regulates the inner world by way of effects on smooth muscle, glands, and the cardiovascular system.

"Autonomic nervous system" was coined by Langley almost a century ago. He was referring to the chain of ganglia lined up, like pearls on a necklace, outside each side of the spinal column. These ganglia contain nerve cell bodies and they lie outside the central nervous system. Langley thought they might have different functions that were independent of the central nervous system; they were autonomous, so they were "autonomic." This view is incorrect, but the phrase "autonomic nervous system" is still used. Langley's autonomic nervous system had three components: an enteric nervous system; a gastrointestinal tract, of which the neurotransmitters are unknown and the multiple possible functions poorly understood; and the parasympathetic nervous system, which opposes the sympathetic nervous system.

The autonomic nervous system regulates the inner world by way of effects on smooth muscle, glands, and the cardiovascular system.

The "sympathetic nervous system" is an ancient phrase that goes back to the time of Galen, whose ideas dominated medical thought from the second century until Harvey's description of the circulation of the blood in 1648. Galen taught that there are spirits in the body: animal spirits, vital spirits, and natural spirits. The animal spirits were distributed by the nerves, which were conduits by which the animal spirits would coordinate functions of the body's organs. These spirits worked in concert with one another, in "sympathy" with one another. The phrase "sympathetic nervous system" antedates the idea of the circulation of the blood by 14 centuries, and no one has produced strong evidence for the existence of the spirits. However, the idea that the sympathetic nervous system plays an important role in coordinating body functions is essentially correct.

In the early twentieth century, Walter Cannon, the American physiologist who coined the phrase "fight-or-flight" and the word "homeostasis," introduced another component to the autonomic nervous system: the adrenal medulla. Cannon taught that the sympathetic nervous system and adrenomedullary systems were a functional unit, which became known as the sympathoadrenal system, the sympathoadrenomedullary system, or the sympathicoadrenal system. This system is an emergency system, not necessary for daily activities, that is required for fight-or-flight emergencies. Cannon was so convinced about this unitary sympathoadrenal system that, in the mid-1930s, he formally proposed that the hormone released from the adrenal medulla (adrenaline) also was the chemical messenger of the sympathetic nerves. He was wrong, and this cost him a Nobel Prize because, in 1946, Von Euler correctly identified the sympathetic neurotransmitter as norepinephrine —adrenaline's "father" in the catecholamine family, which is closely related but not identical—and Von Euler shared a Nobel Prize for that discovery in 1970. The idea of a unitary sympathoadrenal system has persisted to this day. Evidence indicates not only that these are separate components of the autonomic nervous system but also that they are separately dysregulated in at least one condition that is associated with chronic fatigue.

A fifth component to the autonomic nervous system deals with sweat—sweating in response to a gustatory stimulus such as eating chili peppers, to emotional stress, and for thermoregulation. Sweating is a sympathetic function that is mediated by acetylcholine as its transmitter. A person who has chronic fatigue, defined as orthostatic intolerance and orthostatic hypotension from a problem with the ability to synthesize norepinephrine, should be able to sweat normally. Patients who have CFS often have orthostatic intolerance.

In 1946, Von Euler correctly identified the sympathetic neurotransmitter as norepinephrine.

Two forms of chronic orthostatic intolerance are associated with CFS, and patients often have different results in the positive tilt table test. In one result, the patient faints, having developed neurally mediated hypotension and a sudden drop in blood pressure, usually with a relative or absolute bradycardia, which is what constitutes the positive tilt table test. Other patients, or sometimes the same patients, can have an excessive increase in pulse rate and postural tachycardia, with or without subsequent fainting. In one study, regardless of a type of response or whether it was both, the tilt table test was considered positive. Approximately 60 percent of the patients tested with CFS had a positive tilt table test, indicating that orthostatic intolerance, as indicated by the responses to tilt, is common in CFS.

Fainting is associated with two classic features, which indicates the absence of a unitary sympathoadrenal system. The first is a failure of the sympathetic nervous system. Multiple studies have used multiple measures of sympathetic outflow, including direct sympathetic nerve recording showing that a loss of sympathetic outflow, at least within the skeletal muscle and heart, is characteristic of neurocardiogenic syncope (the sympathetic withdrawal). At the same time, everybody knows that people who faint turn pale, which is cutaneous vasoconstriction. Sympathetic withdrawal does not cause cutaneous vasoconstriction. Adrenaline and epinephrine are major culprits—these patients have high adrenaline levels, which is why they are pale. How can high adrenaline levels and withdrawal of sympathetic outflow occur at the same time with only a unitary sympathoadrenal system?

Most but not all patients in a tilt-testing study referred for tilt table testing because of orthostatic intolerance also had a complaint of chronic fatigue. Normally, forearm vascular resistance goes up; in this study, the forearm vascular resistance approximately doubled. Norepinephrine doubled or tripled, which is also normal. Adrenaline or epinephrine goes up by the same proportion, so that the increase in norepinephrine as a fraction of baseline, divided by the increase in epinephrine as a fraction of baseline yields a value of approximately 1.

In a patient with neurocardiogenic syncope tilted, at first the forearm vascular resistance goes up as it is supposed to, but then gradually it creeps down and eventually falls below 1. The forearm is now vasodilated compared to baseline and, within minutes, the patient has had syncope. Norepinephrine and epinephrine both go up normally by the same proportion. As the tilt goes on, norepinephrine increases a little and epinephrine goes up progressively. As a mirror image to the forearm vascular resistance, sympathoadrenal imbalance occurs and adrenaline goes up more than norepinephrine.

To understand the mechanism of syncope, it is necessary to understand what causes sympathoadrenal imbalance and what causes forearm vasodilation, because these events occur before hypotension. In this study, the people who had neurocardiogenic syncope, on average, showed sympathoadrenal imbalance of more than ten times normal. After the syncope, sympathoadrenal imbalance remains and, with recovery, elevated epinephrine persists and helps explain why people feel sick after a positive tilt table test, often for hours or even days.

Between episodes, it is not yet clear whether any abnormalities occur in people who faint a lot, either with postural orthostatic tachycardia syndrome (POTS) or without. Many patients who faint say they do not feel right: “I have fatigue,” “I can’t get up,” “I can’t tolerate heat,” “I can’t eat a large meal,” “I feel disabled,” “I can’t exercise because I feel terrible afterwards,” or “We have this Jacuzzi™ and I don’t know what to do with it.” This predisposition is caused by a failure of the sympathetic nervous system. In a study on sympathoadrenal imbalance, two groups of patients, one with POTS and the other with syncope, differed remarkably in cardiac sympathetic function. This function is assessed by infusing tritiated norepinephrine. To figure out how much norepinephrine is entering

the venous blood of the heart, it is necessary to figure out the extent of dilution of the radioactive norepinephrine as it goes through. The results in patients with chronic orthostatic intolerance showed that, in POTS patients, the cardiac norepinephrine spillover is high; in syncope patients, the cardiac norepinephrine spillover is low. This strong finding is independent of a cardiac event.

The first take-home lesson is that there is no sympathoadrenal system, because it is not a unitary system. These systems are dysregulated independently.

Hans Selye, who introduced stress as a medical scientific idea, defines stress as a nonspecific response of the body to any demand upon it. Because of that definition, the view arose that it does not matter what the stressor is—that giving IL-6 to someone is the same as making someone hypoglycemic. Exercise is an insolent stress and standing up and talking in public is a stress; all stress is the same and a single neuroendocrine stress response occurs and can be studied. Whatever the stressor, the same syndrome occurs and is characterized by adrenal enlargement that translates into activation of the HPA axis, gastrointestinal bleeding, and suppression of immunity. Every stressor has specific and nonspecific components. Selye was only interested in the nonspecific response, that is, a common feature that was shared among all types of stress.

The concept of allostatic load can go a long way to introducing ideas about how chronic stress or stressors can lead to long-term system breakdown.

Chrousos and Gold at the National Institute of Mental Health (NIMH) and the National Institute of Child Health and Human Development (NICHD) modified Selye's theory importantly by introducing the notion of a threshold. Stress or the stress response—the stress syndrome—will be turned on if the intensity of any stressor goes above a certain threshold. That theory can be shown mathematically to be incorrect because it does not account for the data. Pecak published a study in the *American Journal of Physiology* in 1998 that tested and refuted the doctrine of nonspecificity.

What about a homeostatic definition of stress? A homeostatic system is like a house's thermostat system. Afferent information is compared with set points for responding, and the discrepancy is what drives the multiple effectors, leading to relative preservation of levels of the monitored variable. Stress is a condition, a state; in psychology, stress would be called an intervening variable, analogous to an emotional or motivational state. Selye and his students only dealt with the HPA axis. Cannon only dealt with the adrenomedullary model system. Experts in any one of these systems maintain a view that focuses on only one system.

Adrenaline plays a major role in distress. The response to hypoglycemia involves stimulation of the adrenomedullary hormonal system, much more than the HPA axis. Water deprivation is stimulated by vasopressins, the body's main water-retaining hormone. Salt deprivation is stimulated by the renin-angiotensin system; aldosterone is the body's main salt-retaining steroid.

Fight is not the same as flight. A person who is terrified cannot make spit because spitting requires intact sympathetic and parasympathetic nervous systems. Spitting is more than making saliva; it has an instinctively communicated signal value. Fight, flight, fright, and faint are different from one another and have different patterns, and one dependent measure cannot show all these patterns.

Stellar introduced the term "allostasis." To understand allostasis, think about the temperature and humidity in a house. The goal of homeostatic systems is to keep all variables the same. In allostasis, the settings are regulated dynamically and there is no ideal value for glucose, blood pressure, sodium, or temperature. A good example is a house in which the windows have been left open for a year. There is no temperature regulation in that house, and the systems have broken down; to keep the same allostatic setting requires use of the effector systems to such an extent that they have failed. According to McEwen, allostatic load refers to the long-term energy use or system use and damage because of wear and tear that results from some other factor imposed on the system. The concept of allostatic load can go a long way to introducing ideas about how chronic stress or stressors can lead to long-term system breakdown.

How can the sympathetic innervation of the heart be assessed? Sympathetic imaging agents can be used, the best studied of which is 123 I-MIBG (meta-iodobenzylguanidine). More than 600 studies

of 123 I-MIBG have been conducted in the heart to look at sympathetic innervation and function in a variety of diseases. Not a single study has come from the United States (all were conducted in Europe and Japan) because, until recently, 123 I-MIBG was not available here.

A sympathetic imaging agent called fluorodopamine was developed by Dr. Goldstein at the NIH. Fluorodopamine is a catecholamine—dopamine with a positron-emitting isotope of fluorine at the sixth position on the benzine ring. Fluorodopamine works as a sympathetic imaging agent and is removed rapidly from the bloodstream, as are all catecholamines. Fluorodopamine in the interstitial fluid is taken up by a process called Uptake One, which was discovered by Julius Axelrod at the NIMH (who shared the Nobel Prize with Von Euler in 1970). The radioactivity is translocated into vesicles in the sympathetic nerve, allowing a view of the sympathetic nerves that are distributed homogeneously in the heart. In pure autonomic failure, the heart is not visible at all on such scans.

Shy-Drager Syndrome, or multiple system atrophy with sympathetic failure, is associated with orthostatic hypotension and normal plasma and norepinephrine levels; innervation is present. The problem is with regulation of that innervation by the brain. In contrast, in patients with Parkinson's disease and orthostatic hypotension, there is a loss of sympathetic nerves in the heart, demonstrating that Parkinson's is not merely a brain disease and not merely a disease of the nigrostriatal dopamine system; it is also a disease of the sympathetic nervous system.

One way to get at function is by ligand displacement. If a lot of dopamine is being released from a particular terminal, an easily displaced ligand could be injected for the dopamine receptor. A drug, such as amphetamine, that increases extracellular fluid levels of dopamine could then be administered. The endogenous dopamine will compete with the ligand for the receptor and, if the conditions are right, a break in the line for radioactivity will occur if dopamine is being released. The ligand will be displaced; the radioactivity will come out.

This experiment has already been conducted for Raclopride, looking at dopamine in the brain. It could also be done for parasympathetic cholinergic innervation of the heart, because most of the receptors are muscarinic M2 receptors. If a loss of parasympathetic cholinergic terminals were to occur, as is posited from the heart rate variability approach in CFS, then prevention of the breakdown of endogenous acetylcholine by administering a cholinesterase inhibitor would lead to a break in the curve if acetylcholine were present. If acetylcholine were not present to release, then no change in the binding would be seen.

Ligand displacement is an alternative idea for looking at sympathetic and parasympathetic innervation of the heart, as well as general neurotransmitter systems anywhere in the body.

Ligand displacement is an alternative idea for looking at sympathetic and parasympathetic innervation of the heart, as well as general neurotransmitter systems anywhere in the body.

CNS and ANS Responses to Exercise in Patients with CFS

Peter D. White, M.D.

Many patients with chronic fatigue syndrome say they do not feel too bad until they start doing something, and that exercise is the worst thing they can do. The most ideal stimulation test to use in CFS is exercise because exercise exacerbates the illness. One of the most robust findings in the field of CFS is that people with CFS do not like to exercise to exhaustion, probably because they find the effort of exercising much more difficult than when they were in good health.

Exercise, Perception, and CFS

Exercise scientists have used the rating of perceived exertion (RPE) to measure the perception of effort used to exercise. This scale runs from a value of 6 (very, very light) to 20 (very, very hard), and was designed by Gunnar Borg of Sweden. Multiplying each scale value by 10 produces an equivalent heart rate value of 60 to 200.

Many studies show a close correlation between the RPE during exercise and actual physiological effort. In a study by Paul and colleagues, participants were asked to exercise at only 40 percent of their maximum capacity. CFS patients had a much higher RPE compared to the controls, perceiving more effort even at the beginning. Edwards and colleagues compared a larger group of CFS patients with healthy controls. When RPE (y axis) was graphed against heart rate (x axis), the linear curve shifted to the left for the patients, consistent with greater effort perceived with the same amount of physiological work. This result occurs in all exercise studies with CFS and is probably the most robust finding in all CFS research.

We also know that this abnormality responds to appropriate treatment. Fulcher and White administered graded exercise therapy (GET) for 12 weeks to 66 CFS patients who did not have a comorbid psychiatric disorder. Exposure to GET shifted the RPE curve back to the right. Although GET participants experienced increased fitness and increased strength, these were not associated with getting better overall. It appears that mere exposure to graded exercise is what normalizes RPE and therefore GET is more a "brain treatment" than a physical training treatment.

Apart from fitness and strength, research has shown that several brain-mediated elements help determine a greater sense of effort with exercise:

- *Sleep*. Individuals who have trouble sleeping.
- *Mood*. People who are anxious or depressed.
- *Somatic perceptions (introspections)*. People who are more aware of their bodies.
- *Introversion*. Those individuals who are more internally oriented.
- *Emotionality*. Individuals who generally react emotionally to events.

In a 1990 study by Riley and colleagues, participants with CFS were asked to estimate how fit and well they were before they developed CFS. They were compared with people who were currently healthy and with people who had irritable bowel syndrome. People with CFS reported that their premorbid fitness and health were even better than healthy people and people with irritable bowel syndrome. Either CFS individuals are fitter, stronger, and healthier before they get ill or their perceptions of normality change during the time they are ill.

The Autonomic Nervous System During Exercise

People with CFS are as unfit as healthy but sedentary people. Although CFS patients are as unfit as sedentary people, sedentary people tend to be sedentary most of their adult lives; people with CFS have become sedentary. Wessely, Sharpe, and others have suggested a cognitive behavioral model of CFS: it is well known within the field that dysfunctional illness

beliefs and avoidant coping perpetuate both the symptoms and disability of CFS. One of the beliefs of CFS patients that predicts outcome is that exercise is dangerous or damaging. It is possible that this leads to fear of exercise, an exercise phobia. CFS patients not only avoid exercise, but also they may avoid it because of a fear response that may be autonomically mediated.

If CFS is a classically conditioned exercise phobia, situational fear, avoidance, and autonomic nervous system arousal could be expected. CFS patients may avoid exercise and express a fear of exercise because of a rational fear of having a relapse. To test for excessive arousal, we measured electrical skin resistance during an ordinary day and during an exercise test. The 42 study participants with non-comorbid CFS were compared with healthy sedentary controls matched in various ways. During the second day of the study, participants exercised on a treadmill and researchers measured activity with an ankle accelerometer, heart rates, and galvanic skin responses (GSR) (the measure of electrical resistance across the palm, which is related to the level of physiological arousal). No difference in GSR was seen between people with CFS and healthy sedentary controls, both during an ordinary day and during exercise; they were no more aroused than controls.

CFS probably is not an exercise phobia. However, the severity of both fatigue and disability was associated with illness beliefs, deconditioning, perceived exertion, and sensitivity to physical symptoms. Prospective studies also suggest that fitness, beliefs, and sensitivity are predictive of outcome in CFS and are not merely associated with it.

Exercise, the CNS, and Hormones

The effects of exercise on the brain are many: the brain becomes more active and exercise elevates concentrations of the neurotransmitters serotonin, dopamine, and norepinephrine, as well as beta-endorphin, prolactin, ACTH, and cortisol. More intriguingly, exercise increases brain-derived neurotrophin, a growth factor for the brain. Acute exercise has a small enhancing effect on sleep, whereas persistent inactivity causes poor quality sleep. Women in general have longer, but less good-quality sleep and women are generally less fit. It is possible that these two factors may help to explain why women get CFS more often than men. Exercise is a stressor for people who are physically unfit, with excess cortisol response, while inactivity downregulates cortisol receptors. Being unfit therefore affects hormones and produces an excessive stress hormone reaction. However, it may also be true that people who have a generally impaired HPA axis become fatigued when unable to exercise. In a patient with CFS, Sharma and colleagues showed that the excessive prolactin response to buspirone reverted to normal after graded exercise therapy, suggesting that this effect may be secondary to inactivity and thus reversible in some people.

CFS probably is not an exercise phobia.

Shephard has reviewed how exercise induces an inflammatory response, depending on endurance and exertion, and how exercise induces both a pro-inflammatory and an anti-inflammatory cytokine cascade. This response is exaggerated in unfit people. Interleukin-6 is produced by both glial and muscle cells during exercise. Could CFS be caused by an excessive cytokine response to exercise? Patients with CFS have normal serum cytokine levels, on the whole, but not all cytokines have been studied. Post-exercise cytokine levels are similar to pre-exercise levels, but the "exercise" involved may have been insufficient. Jones and colleagues recently used a bicycle exercise test and found that a complement component increased after exercise in CFS patients more than healthy controls.

TNF-α not only increases with exercise, especially for unfit individuals, but it also increases with stress. In a study just published, Steptoe and Owen looked at healthy people having a stressor (a psychological test) and found that those who had the highest heart rate response to the stressor—those who were the most stressed—also had the highest TNF-α response. This result indicates that exercise and mental stress may induce similar cytokine responses; two different stressors may have the same physiological and pathophysiological effect. This might explain why patients with CFS respond poorly to both physical and mental stressors, although the evidence is so far not available.

Abnormal Introspection in CFS

Repeated studies that have compared subjectively reported disability with objectively measured activity in people with CFS show a discrepancy between them. CFS patients complain about inability to concentrate and problems with memory but, when tested, the results of cognitive testing tend to be essentially

normal. There are discrepancies between reported abilities and objective ability tests in activity, effort with exercise, sleep quantity and quality, and cognition. A study published last month by Smith and Sullivan suggests that beliefs are part of that discrepancy. These authors gave benign chemicals blindly to people who had CFS and who also believed they had multiple chemical sensitivity. Individuals who *believed* they were getting the chemical performed poorly on cognitive tests, whereas there was no difference in cognitive function between those who actually received the chemicals and those who did not. The belief determined the brain reaction to the task more than the chemical itself; the perception was more important than the chemical.

Hypotheses To Be Tested

Four hypotheses can be gleaned from these studies:

1. Post-exertional fatigue or malaise is caused by an excessive cytokine cascade, itself related to altered activity, deconditioning, and sleep disturbance (see Figure 1).
2. This reaction may be triggered by certain infections, but is maintained by a conditioned response to exercise (see Figure 1).
3. The symptoms of CFS are caused by CNS mediated interoceptive hypersensitivity, in a similar way to chronic pain syndromes, perhaps through classical conditioning.

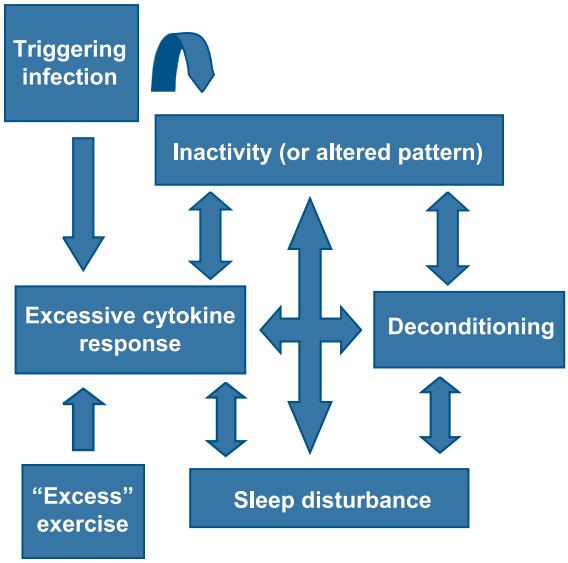


Figure 1

4. This introspective hypersensitivity is related to poor sleep, inactivity, and deconditioning, through consequent neuroendocrine and cytokine links (see Figure 2).

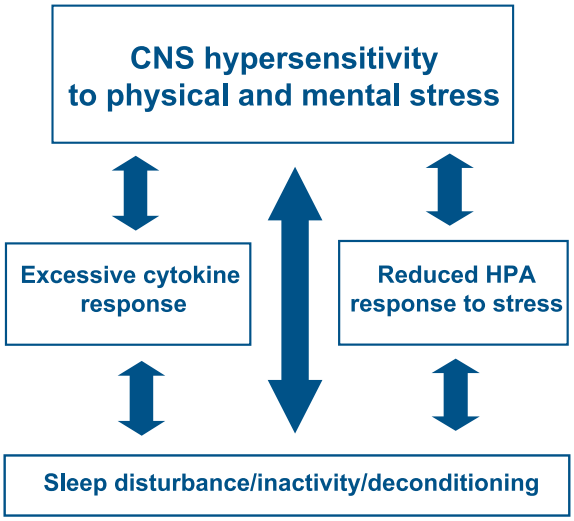


Figure 2

Conclusion

A biopsychosocial model is more useful than a biomedical model in understanding CFS. The neuroimmune-endocrine abnormalities of CFS may be maintained by a conditioned response to activity and exercise (a testable hypothesis). This may explain why a gradual return to avoided activity, common to both graded exercise therapy and cognitive behavior therapy, helps the majority of patients with CFS.

Discussion

Dr. Morens: If people with CFS are conditioned to be physically fit, the heart rate goes down. Does their perception of effort change after conditioning? After conditioning, there is a discrepancy between heart rate and actual performance. Does the perception of effort correlate with the heart rate or with the actual work done?

Dr. White: Both occur. Although RPE shifts back and a training effect occurs, no association was found between patients feeling better and actual physiological improvements of conditioning. It may be that this is CNS desensitization to the perception of effort, rather than simply being a training effect.

Dr. Buchwald: Do you know of any studies that looked at perceptions such as vision, hearing, or taste in which standardized perception measures are available?

Dr. White: One study on visual sensitivity, which some CFS patients report, did not find an association with physiological evidence for visual sensitivity. This area is unexplored.

Corticotropin-Releasing Hormone, Insomnia, and Depression

Gary S. Richardson, M.D.

Insomnia is a broad term that is generally defined as a complaint of inadequate sleep despite adequate effort. From a clinical or treatment standpoint, insomnia is classified in two broad categories: secondary and primary. Sleep disruptions associated with CFS and other disturbances are secondary insomnia. Anything that causes disturbed sleep and sleep complaint is secondary insomnia, including chronic pain, a variety of neurologic conditions, or cardiovascular disease. Primary insomnia is a diagnosis of exclusion. As investigators try to characterize the pathophysiology, the distinctions become spurious: many of the effects and symptoms associated with primary insomnia can be found in people who have secondary insomnia. Depression is a good example: 80 to 90 percent of people who are depressed have sleep disturbance. Even though that may be a secondary insomnia, many features of it are characteristic of primary insomnia.

Primary insomnia is a common disorder. About 10 percent of adults over the course of a year will have significant insomnia and a large percentage of those are primary. There is gender- and age-dependent prevalence: insomnia increases with age and is more common in women. A clear relationship to stress exists, in both precipitation and ongoing insomnia complaints. It has a chronic, recurrent course: data indicate that people with significant primary insomnia, lasting 2 weeks or longer, have a lifelong condition.

Insomnia is linked epidemiologically to major depression. Data indicate that people who have significant primary insomnia and no other symptoms have a much higher risk of developing major depression in 3 to 4 years. People who have one of the major psychiatric disorders are likely also to have insomnia.

Physiologic hyper-arousal is specific to primary insomnia, which may be relevant to CFS. Patients who complain of insomnia of significant duration typically experience insomnia for at least 3 months. Several lines of evidence from different individuals support hyperactivation of the sympathetic nervous system, also called autonomic arousal. A study by Bonnett shows that basal metabolic rate is higher in primary insomniacs relative to age-matched controls. Relevance to sympathetic nervous system activation may be weak, but data from Lushington shows that a group of insomniacs compared to a group of controls had higher body temperature, independent of sleep disruption that characterizes that population.

Heart rate and the low frequency/high frequency ratio are higher in patients with primary insomnia. Data from Stepanski indicate that, at any given point for the control state and for the sleep state, heart rate is higher in patients with primary insomnia. Studies of spectroanalysis of R-R (electrocardiogram R wave) intervals show that sympathetic activation occurs to a greater degree in patients with primary insomnia than in normal controls.

Physiologic hyper-arousal is specific to primary insomnia, which may be relevant to CFS.

In a study by McClure on catecholamine data, ten patients with primary insomnia, in 24-hour urine collections at baseline, were shown to have significantly higher levels of norepinephrine and indistinguishably higher levels of epinephrine. Characteristic of much of the research in the field is that baseline values appear to be only mildly affected. (Vgontzas and colleagues recently showed, with 24-hour urinary norepinephrine levels, a correlation between the degree of norepinephrine elevation and the severity of insomnia.) McClure used a modification of the Trier Social Stress Test, which measures the remarkable activations, principally of cortisol, in anticipation of a public speech—a broadly applied psychological stressor. In a 3-day paradigm, subjects were admitted to the

laboratory and told they would be part of a study on stress levels and sleep. Ten subjects were primary insomniacs and seven were normal controls. Without forewarning, subjects were approached on the evening of the third night and asked if they would be interested in participating in another study. If they agreed to this second study, they were taken to a large auditorium the evening before the third night and were given three difficult speech topics to choose from, with minimal supporting papers. They were then left alone for an hour, and then brought back to bed. Many of these subjects did not sleep the entire night. They got up the next morning and were taken to a conference room with five psychology interns sitting around a conference table with a video camera, and were then asked to give their speech.

The Multiple Sleep Latency Test (MSLT) is a measure of how sleepy a person is. It is an objective recording of the polysomnographic parameters, to record the exact moment at which a person falls asleep under standardized conditions. The longer it takes to fall asleep, the more alert the person is. MSLT urine data after the public speech showed that epinephrine levels were unchanged in the normal population but changed significantly in the insomniacs; these people were unimpressively different at baseline despite having relatively severe insomnia.

As a result of McClure's study, the model under consideration is that this is a group with a hyperactive stress response and, in particular, an inability of that response to restore normal levels in stress indexes after the stressor is removed.

In another study using the MSLT, Stepanski looked at 45 normal individuals and 70 insomnia patients who had the same bedtimes but not the same amount of sleep. The MSLT was higher in the insomniacs relative to the normals even though they got less sleep; this shows that the MSLT is sensitive to a full-day physiologic hyper-arousal and that the elevated levels of arousal translate into longer sleep latencies.

In McClure's paradigm using the MSLT before and after the public speech, normals start off worse but insomniacs and normals show the same decrease in sleep deficiency in response to the public speech. What predicts the percentage drop is less related to insomnia than to how much the subject dislikes public speaking. Both controls and insomnia patients have substantially worsened sleep the night before the speech. As soon as the speech was over, participants were sent back to their rooms to watch television. The normals collapsed and showed increased levels of sleepiness. However, the MSLT scores of the insomniacs increased; they were more physiologically aroused by this experience even after the experience was over. This concept is crucial and central to the pathophysiology of primary insomnia.

Sympathetic activation seems to alter perception of what is occurring.

Another study investigated whether or not this physiological arousal is primary or secondary to the insomnia. Data from other studies suggest that chronically disrupted sleep produces physiologic arousal: as a tool to explore hypertension, Gordon used acute dietary salt restriction, which produces an activation of the sympathetic nervous system with increases in catecholamines, and in 1983 Ditiello used acute dietary salt restriction to produce sleep disturbance in normal individuals, concluding that it made them look like they were old. With these and other studies as background, Dr. Richardson studied 11 healthy young adults for 6 days and nights in the sleep lab. Subjects were fed a regular hospital diet on day zero (greater than 100mEq of sodium a day), and then they were given a very low sodium diet of less than 17mEq for days 1 through 5. There was some concern about occasional episodes of orthostatic hypotension in this setting; therefore, vital signs were monitored throughout. Norepinephrine did not increase significantly. The assumption that all Americans have a high-salt diet may be a fallacy, so a new study has been undertaken in which all participants are salt-loaded up front.

A modest increase in norepinephrine levels was seen, and some individuals had significant increases in norepinephrine levels. All participants showed a significant decrease in sleep efficiency and a significant increase in the time it took them to fall asleep. In addition, the amount of lightest, least restorative sleep (Stage One) also increases with salt restriction. Although not statistically significant, there is a correlation between the degree of norepinephrine elevation and these measures.

One of the cardinal tenets about primary insomnia is that insomniacs see their insomnia as much worse than it really is. In this study, normal individuals started out being reasonably accurate at estimating

sleep latency, number of wakes, and the quality of their sleep at baseline. However, all of them registered increases in what they thought would happen that were much greater than what was actually happening, particularly in sleep latency measures. One of the conclusions from this study is that sympathetic activation seems to alter perception of what is occurring. The modest increase in sleep latency, despite the sleep disruption, is reminiscent of primary insomnia.

Assuming that the responsiveness of catecholamines is abnormal in insomniacs, one of the implications is that the hypothalamic-pituitary-adrenal (HPA) axis should be affected. We will attempt to implicate the corticotropin-releasing hormone (CRH) neuron in the hypothalamus, one of the tests of which will be the appearance of abnormalities in the HPA axis.

Whether HPA function is abnormal in primary insomnia is the focus of Penn State researchers who are working on a similar model. They have found a significantly higher basal cortisol level without any stimulation in patients with primary insomnia versus normal controls. Vgontzas showed that patients with worse insomnia had higher urinary-free cortisol. We have begun a study that uses the Dex CRH Test in which subjects are given dexamethasone the night before the test to create comparable degrees of suppression; their systems are then stimulated with exogenous CRH at time zero. To date, ten normals and three patients have been studied. The first three subjects do not show escape at baseline but do show an impressive increase in ACTH, much more than cortisol, in response to Dex CRH. In combination, these data support the idea that the HPA axis and the sympathetic outflow are abnormal in insomnia patients.

Stress precipitates or perpetuates primary insomnia. Several longitudinal studies have shown that stress is an important factor in primary insomnia. If it is true that CRH is important to depression, then the fact that insomnia is linked both epidemiologically and syndromally to depression supports the idea that CRH is involved in insomnia. In several preclinical animal studies, CRH was shown to mimic many of the symptoms associated with primary insomnia. Data support the idea that throughout the brain locations for CRH, this peptide serves a relatively singular integrative function—it seems to be involved in mediating or integrating the stress response. When CRH is injected around the brain stem, EEG desynchrony is produced, which can be characterized as wakefulness. Studies with antagonists show that CRH increases wakefulness at the circadian wakefulness bays and, in particular, in the setting of stress-induced insomnia.

Opp and colleagues conducted a study of stress-induced insomnia in which socially isolated rats showed a decrease in the amount of sleep. In group-housed individuals infused with the CRH antagonist, no net effect was seen. However, in stressed animals, the stress-induced decrease in sleep and the alpha-helical CRF (the antagonist) restored sleep levels to near normal. Several active investigations are now using CRH antagonists in humans to find out if these drugs can inhibit stress-induced insomnia without producing sedation when stress is not present.

Stress precipitates or perpetuates primary insomnia.

Pivagabine is a drug associated with a decrease in CRH release. In individuals with insomnia in the setting of psychiatric disease, pivagabine is associated with a substantial improvement in insomnia independent of the change in the underlying psychiatric disease.

Several researchers have suggested that catecholaminergic tone in particular (and sympathetic outload generally) has an ideal set point, which is not zero and not maximal but somewhere in between. A convergence occurs when looking at CNS effects of pushing that system away from its set point in either direction. One of the challenges is to understand how researchers can be working in what seem to be opposite directions and yet end up with many similar outcomes.

Discussion

Dr. Crofford: I am intrigued by perception versus reality, a disconnect being described by several people. What kinds of chemical mediators or cortical sites might be creating this? Some people have talked about norepinephrine as potentially mediating this and some have talked about CRH. Does anyone know the neuroanatomical localization of perception, which seems to cross many different symptom domains? Does anyone know whether or not it is norepinephrine-predominant or CRH-predominant? A perceptual axis is presumed in cortical sites.

Dr. Park: Explicit recollection is largely in the frontal cortex. It is possible, but not necessarily plausible, that CFS patients have some kind of frontal dysfunction. Instead of relying on what they explicitly remember, they rely on what they feel or what feels familiar. People are talking more about beliefs than about perception. The discussion largely revolved around people saying they believe something is true, and that belief is just disconnected from their behavior. A belief is different from a perception. A perception should have a sensory analog in the visual cortex or the auditory cortex.

What presenters have shown today is malleability in neurochemical systems, but no equivalent behavioral work has been presented thus far. More responsivity to emotional stimuli would be expected at the neurological and behavioral levels in a CFS patient who exhibits neurobiological reactivity. Patients have been well characterized neurobiologically, but not well characterized behaviorally.

Dr. Clauw: This spectrum of illness may well be a problem with interoception and more than just pain or other symptoms. People with CFS or fibromyalgia have shown a poor relationship between, for example, their self-reported physical activity and objective measures or between self-reported cognitive function and what is measured. That result is also true of controls—many of these disconnects are equally true in control populations.

Bud Craig is focusing on the anterior insula as being an area of interoception. There is a great deal of converging evidence from both animal models and human trials with, for example, dyspnea and other unpleasant sensations. The neurochemistry is not as well characterized as the neuroanatomy, but many researchers are focusing in this area as possibly being an anterior septa varum.

Dr. White: At Kings, a group of researchers used an activity intervention after mononucleosis to prevent abnormal fatigue for 6 months; results show a 50 percent drop in fatigue in 6 months. It was only a pilot study, but 69 patients were studied. If a grant is won, a larger study will begin. Patients were encouraged to become more active in a graded way within a month of the onset of their infectious mononucleosis. In 6 months, the complaints of fatigue were reduced by one-half.

Dr. White: The two first studies of CBT for CFS were successful. In Sharpe's study at Oxford and Wesley's study at Kings, there was no change at the end of treatment compared to the two control groups (one receiving usual care and the other controlling for therapist time). However, 8 months later as they became behaviorally more active, desensitization occurred and there was a change from the placebo groups. Symptoms and disability both improved. The beliefs had changed but their behavior, symptoms, and disability had not changed. Only when subjects were persuaded to become more active did they get better.

What presenters have shown today is malleability in neurochemical systems, but no equivalent behavioral work has been presented thus far. More responsivity to emotional stimuli would be expected at the neurological and behavioral levels in a CFS patient who exhibits neurobiological reactivity. Patients have been well characterized neurobiologically, but not well characterized behaviorally.

Session Three: Cytokines, Sleep, and the CNS

Chair: Harvey Moldofsky, M.D.

One of the recurrent themes related to chronic fatigue syndrome heard throughout this workshop has been poor sleep—problems falling asleep, staying asleep, or waking feeling unrefreshed. What happened, or the perception of what happened, during the previous night affects how people feel the following day in terms of fatigue, cognitive problems, and autonomic disturbances. Apparently, there is something compelling about sleep, as well as how the sleeping and waking brain is related to regulatory functions involving cytokines, neuroimmune, and neuroendocrine functions.

Genetics of Sleep Regulation

Linda A. Toth, D.V.M., Ph.D.

Sleep is a ubiquitous biological phenomenon. It has been described in all mammalian and avian species studied to date, and sophisticated genetic studies are now beginning to explore the genetic basis of sleep in animals such as Drosophila, nematodes, and fish. The field of genetic regulation of sleep is expanding rapidly.

Humans spend about one-third of their lives asleep, and some animals (for example, cats) spend two-thirds or more of their lives asleep. Despite all the time devoted to sleep, ideas about what sleep does for the body remain controversial, with lively ongoing debate about questions as fundamental as whether the functions of sleep are understood. The field of sleep has made considerable substantive progress in the past 10 years, developing from what was considered a soft science to what is now seen as cutting-edge brain neurobiology. Genetics have helped move the field in this direction.

Sleep problems and alterations in sleep are not confined to people with chronic diseases. Many people suffer at some time in their life from a sleep problem, either inability to stay asleep or inability to experience refreshing sleep. For some patients with chronic diseases, these problems can become debilitating. This is being recognized increasingly as treatments are developed for diseases, such as hepatitis and HIV, once relatively acute and potentially fatal but now persistent and chronic. In many of such patients, perhaps because of their treatments, among their major complaints are problems with sleep or nonrestorative sleep or fatigue.

In the state of infectious disease, a putative reason for going to sleep is apparent, at least on an anecdotal basis. Many people have experienced acute infectious diseases, such as a cold or influenza, and during these times most have felt either an increased desire to sleep or, depending on the stage of the disease, an inability to sleep. There is an almost intuitive feeling that sleep has some curative or protective properties. Most people have said or heard the following: "You had better get some sleep or you will never recover" or "If you do not get some sleep, you will become ill." There is a sense that sleep and the immune response are related and that sleep can aid recovery from infectious diseases.

In 1988, there were few empirical data to show that sleep changed in any way over the course of an infectious disease. Since then a body of data has accumulated showing that sleep does change during infections. Some are self-reports in human studies but some have also been followed up polysomnographically. In animals, researchers rely on polysomnographic reporting to indicate whether the animals are awake or asleep.

Sleep does change during infections.

A wide variety of infectious challenges have been tested and found to cause robust changes in normal spontaneous sleep patterns. For example, in the rabbit model, bacterial, fungal, influenza, viral, and even protozoal infections can influence the immune response and the sleep response. These responses are modulated by the basal immunologic state of the animal; environment, hormonal status, circadian cycle; and other features affect the type of response.

We have worked extensively with mice infected with influenza virus. In the basic experimental design, the mice undergo surgery and are implanted with electroencephalogram (EEG) and electromyogram (EMG) recording electrodes, so that recorded EEG data can be used to score sleep. After a 2- to 3-week recovery period, their sleep is studied for 1 to 2 days and then they are inoculated intranasally with influenza. This model gives the mice an upper

respiratory tract infection and a pneumonia from which they would typically recover in 7 to 10 days; it is analogous to a severe human cold, except that the mice also get pneumonia. Usually the mice are euthanized 3 to 4 days after infection, so that various parameters can be measured.

One of the strains of mice utilized in these investigations is the inbred C57BL/6J; those inbred mice are selectively brother/sister-mated for at least 20 generations. Studying an inbred mouse strain is considered analogous to an unlimited twin study with many genetically identical animals. Working with a complicated phenotype such as sleep, this makes it possible to get an average phenotype for a fixed genotype. The hundreds of strains of inbred mice are part of the armamentarium to try to dissect the genetic basis for many complex traits, such as infection-related changes.

In their basal sleep patterns, C57BL/6J mice showed a marked circadian rhythm, sleeping more during the light phase and becoming nocturnally active when lights were off. In mice inoculated with allantoic fluid, no marked changes were observed in sleep patterns. However, within about 18 hours, mice inoculated with influenza virus began to show a marked increase in the amount of time they spent asleep. The increase was most pronounced during the dark phase when the mice would normally be awake, representing loss of the circadian rhythm of sleep. The mice essentially slept the same amount of time all the time—behaviorally equivalent to a person getting sick with a cold or flu and staying home from work and sleeping all day. This is essentially equivalent to an excessive daytime sleepiness response in people.

The many strains of inbred mice exhibit substantial genetic differences. The C57BL/6 strain and the BALB/c strain vary in a number of immune response parameters. For example, C57BL/6 mice tend to show a cytotoxic T1 cell response more predominately, while BALB/c mice are T2 responders. The two strains have different major histocompatibility complex haplotypes. IF1 genes, which control interferon production and respond differentially in a variety of paradigms, are different. Any of these differences—and potentially many others—could theoretically influence how these mice sleep after microbial challenge.

Like C57BL/6 mice, infected BALB/c mice showed a loss of the circadian rhythm of sleep; however, rather than developing increased sleep, they showed reduced sleep during their normal period of somnolence. When they should have been resting or sleeping, they exhibited instead an insomnia-like phenotype. Immunized C57BL/6 mice rapidly cleared the virus and their sleep patterns quickly returned to normal, whereas immunized BALB/c mice still showed a tendency to sleep a bit less than before.

These characteristics are shared by many inbred strains of mice. A survey of nine strains found several that, like BALB/c, showed a reduction in sleep during the light phase of the cycle during infection. Other strains, such as C57BL/6, showed the daytime sleepiness phenotype. These two patterns of change in sleep response to one particular infectious challenge seem to be fairly common across a variety of different inbred strains of mice.

Influenza is not the only infection with this effect. In mice with nephritis caused by *Candida albicans*, changes occurred in both slow-wave sleep and REM sleep. In a model of immune-mediated hepatitis caused by injecting mice with Concanavalin A, strain differences in sleep also occurred. The sleep patterns of A/J mice did not change, BALB/c mice showed a moderate increase in sleep time, and C57BL/6 mice showed an even greater increase.

These two patterns of change in sleep response to one particular infectious challenge seem to be fairly common across a variety of different inbred strains of mice.

Two approaches have been used to study the cause of these responses. The first is gene-driven, with the general hypothesis that a particular gene influences the expression of the sleep phenotype. The tools used to study the impact of known or potential sleep regulatory substances on the sleep pattern are often induced or spontaneous mutant mice, drugs, or antibodies. One example is the study of interferon-α as the candidate substance for influenza-related enhancement of sleep. Because it is known to induce sleep, interferon-α is a potential mediator

for increased sleep during infection. It is the classic antiviral cytokine known to be produced in large amounts during at least the early stages of viral infection, and the IF1 gene in mice genetically controls it. C57BL/6 mice have the allele for high production so they make more interferon, which could cause excessive sleep; BALB/c mice have the allele for low production and therefore could have impaired sleep.

Whether the IF1 allele causes these divergent responses can be tested in congenic mice strains, which are bred to have the genetic background of one strain with a small genetic segment that is selectively bred into the background strain from a donor animal. In this study, a strain of congenic mice was developed with the H28 histocompatible antigen from BALB/c mice bred onto the C57BL/6 genetic background, the result being a mouse that is 99 percent C57BL/6 but has one genetic segment from BALB/c that also contains the IF1-low allele. When tested, the IF1-low allele was not found to influence the sleep pattern in the model. The C57BL/6 mice with the high allele showed increased sleep, and the C57BL/6 mice with the low allele also showed increased sleep. Neither group looked like the BALB/c mouse, indicating that the interferon allele is not critical to the high-sleep phenotype in this model.

When mice were injected with Newcastle disease virus (NDV), the C57BL/6 mice again showed high-sleep phenotype, the BALB/c mice still showed a relatively low-sleep phenotype, and the response of the congenic mice was similar to that of the BALB/c mice. In this case, the IF1-low allele did seem to be correlated with the sleep response, allowing the conclusion that the role of the IF1 gene depends on the challenge. The IF1-high allele did seem to promote NDV-induced sleep but did not influence influenza-induced sleep, an indication that other factors must mediate the hypersomnolence or hyposomnolence seen in mice with influenza infections. This series of studies illustrates how specifically bred mice can be used to determine the contribution of a specific gene to a complex behavioral phenotype.

With interferon-α eliminated, one strategy is to continue examining factors that could influence sleep, for example, known somnogens such as IL-1 and TNF, and test them individually in order of priority to determine whether they influence the phenotype. Another strategy is a genome-wide search, which is essentially phenotype driven and looks for the genes that affect phenotype expression. Instead of focusing on one specific gene or substance, the focus is on the phenotype, without a priori assumptions about mechanisms. The genome-wide search can be conducted in a variety of ways, including random mutagenesis, microarrays, and proteomics. These searches can survey the entire genome for differences related to a particular condition or phenotype in an animal or human.

Our approach to genome-wide searching used linkage analysis in recombinant inbred mice. These mice are produced by crossing two progenitor strains that differ in some particular trait; the offspring are then inbred through 20 generations. At 20 generations, each mouse is essentially homogeneous for all the alleles. Each has a random fixed assortment of all the parental alleles, allowing determination of average genotypes for each fixed phenotype. These mice are valuable tools for mapping complex traits, and many strains are commercially available.

This series of studies illustrates how specifically bred mice can be used to determine the contribution of a specific gene to a complex behavioral phenotype.

The first step of the mapping study was to determine the phenotypes of each strain being studied. The next step was to look for correlations between the phenotypes and the distribution of alleles at marker loci, and then the task was to estimate the probability of obtaining those correlations. We used this procedure to analyze 13 recombinant inbred strains of mice derived from C57BL/6 and BALB/c parents. This analysis showed a strong linkage of the light-phase phenotype of reduced sleep—essentially, the insomnia phenotype—to a certain region of chromosome 6.

Quite a few genes that might influence expression of this trait were found in the region of chromosome 6. CD8-α is a T-cell antigen that influences the immune response. The ADCYAP receptor has been related to REM sleep regulation. Other candidate genes were neuropeptide Y, growth hormone-releasing hormone receptor, and CRH receptor 2. This genome-wide approach results in a short list of strong candidates. Instead of needing to study 100 candidate genes that might influence a trait, investigators can focus on only a few.

Thus, strategies for identifying sleep regulatory genes in mice are at least dual: the genome-wide search can allow identification of candidate mechanisms for complex phenotypes, followed by a gene-focused approach that can test the contribution of potential candidates. Mice are not the same as humans, but animal studies provide suggestions on how to move forward and what might be useful to study. One example is pinpointing orexin as the genetic lesion that causes narcolepsy in dogs. This was done by Minot's group in 1999 by genotyping a population of dogs that spontaneously displayed narcolepsy and conducting a genetic analysis similar to what might be done in a human population. The work in dogs was corroborated 2 weeks later by a group working in parallel using a mouse with some part of the orexinergic system knocked out; they saw narcoleptic phenotypes in mice. The mice would walk in the cage and suddenly fall over in a cataplectic state, and within a few minutes get up and walk around again. This was revolutionary for the field of sleep medicine because it has tied in a particular genetic abnormality to a sleep phenotype. In one study, as many as 90 percent of human narcoleptics had a deficiency in orexin. It is not likely that this gene would have been examined for relation to a sleep disorder had not the genetic mechanism first been elucidated in an animal model. Thought to be involved in food intake, orexin was initially investigated in the knockout mice to study energy homeostasis, and it suggested a link between energy balance and regulation of sleep that has spurred a remarkable number of investigations in the sleep field.

These types of genetic influences can also be detected by looking at spontaneous sleep apart from sleep disorders. Rab3a is a gene involved in synaptic plasticity; about a year ago it was reported to be involved in not only circadian rhythms (shortening of the circadian day) but also in sleep homeostasis (the rebound response to sleep loss). Rab3a was known but researchers did not connect it to a sleep homeostatic mechanism until a random mouse mutagenesis study detected altered circadian rhythms, and then found that the defect in the random mutagenesis was probably in the Rab3a gene—highly suggestive that the Rab3a gene is involved in sleep regulation. Many other studies have shown that various spontaneous and induced mutations are associated with the regulation of spontaneous sleep in a variety of animal models, and some of these have been extended into human studies using various challenges.

Fatigue is more challenging to describe in animals than is sleep. In animals, sleep can be measured but no simple way to measure fatigue has been developed; people can report fatigue but animals cannot. Increased sleep may suggest fatigue, because sleep can be a response to fatigue; an organism—animal or human—might sleep more because it is tired all the time. However, inadequate sleep or inability to sleep soundly may also cause fatigue. Using locomotor activity as a measure of sleep or fatigue can also be confounding in many cases. For example, an organism could be too fatigued to move much, but might still be unable to sleep or fall asleep. Using voluntary activity such as wheel running might work and has been tried, but such work remains preliminary.

In one study, as many as 90 percent of human narcoleptics had a deficiency in orexin. It is not likely that this gene would have been examined for relation to a sleep disorder had not the genetic mechanism first been elucidated in an animal model.

Influenza-infected C57BL/6 and BALB/c mice that have been immunized illustrate the complexities of these studies. The C57BL/6 mice quickly return to their normal sleep patterns, while the BALB/c mice show slightly less sleep. Locomotor activity and temperature patterns, however, show two different responses. The C57BL/6 mice resumed their normal sleep patterns, but they remained hypoactive and hypothermic (mice with infections tend to become hypothermic rather than febrile). The C57BL/6 mice took a longer time to recover completely from the flu, while the BALB/c mice recovered in a day. Speed of recovery may be related to a specific genetic response.

In studying fatigue, the basic question is what constitutes an appropriate phenotype. Only when an appropriate phenotype for fatigue is described can mechanisms be addressed, either by looking at candidate gene approaches or by using genome-wide approaches. In some cases, sleep may be an appropriate substitute for fatigue, but researchers need to be more inventive to arrive at accurate methods for assessing fatigue. The many different

types of fatigue that have been described in humans may or may not have correlates in animals.

It is hoped that identifying some of these genetic mechanisms will help identify the pathophysiologic causes of fatigue, excessive sleepiness, and nonrestorative sleep in a similar manner to the recognition of the role of the orexin gene in narcoleptic dogs. With this knowledge, the next step will be to develop interventions that will help control or prevent these symptoms, just as orexin has become a new potential therapeutic intervention in treating narcolepsy.

Discussion

Dr. Buchwald: How much variability is there in sleep within a strain in terms of length or other parameters, and has there been any research to account for differences seen in sleep, given that these animals are genetically similar?

Dr. Toth: Some inbred strains diverge a great deal in their normal sleep patterns. Variation within strains also depends on the strain; some seem to be more variable than others. Strains that vary more may be more sensitive to factors in the environment, such as temperature fluctuations or noise. About 35 percent of the variation can be accounted for genetically. Because of the variation within strains, characterizing a sufficient number of animals is needed to get an average phenotype for each strain.

Dr. Sternberg: Are there sex and gender effects?

Dr. Toth: I am not aware of any researchers who have looked at that in mice. Certainly there are such differences in rats in terms of sleep. I have always used male mice and so has everyone else I am aware of, but I would be astounded if there were not also gender differences in rats.

Dr. Sternberg: What are the effects in rats? What are the sex differences?

Dr. Toth: Female rats change their sleep patterns as a function of the estrous cycle, which is why I have hesitated using them. I have never compared females to males. The males seem to be more consistent from day to day. However, in females, the estrous cycle and pregnancy cause changes in sleep patterns.

Cytokines–Neurotransmitter Interactions and the Regulation of Arousal State

Mark R. Opp, Ph.D.

Research suggests the existence of a biologic basis whereby responses to immune challenge can be transduced into alterations and behavioral outcome measures, specifically, alterations in sleep/wake behavior. The questions to be addressed are the functional questions about why individuals sleep and why people sleep differently when they are sick. Most individuals have personal experience with the changes in cognitive well-being and behavior through the course of an infectious challenge.

In the course of an acute infection, the immune system detects the pathogenic invasion, and a variety of physiological and behavioral changes occur. The hallmark manifestation is the onset of fever. If the systems and processes induced by the pathogen function effectively and efficiently to inhibit the growth of the pathogen and return an individual to health, the infection will be resolved and life-as-usual resumed.

In regulation of sleep/wake behavior, every major neurotransmitter system defined in the brain is involved at some level.

The question of why people sleep differently when sick can be addressed in two ways. A functional perspective considers whether this aids in recuperation and whether people recover more quickly because of changes in sleep. A more mechanistic perspective considers what happens to convey information from the peripheral immune system to the brain that ultimately results in changes in behavior and physiological processes. While functional questions are important and ultimately should be addressed, elucidation of mechanisms involved in altered behavior through the course of immune challenge is also critical.

Many systems are involved in sleep and other complex behaviors. There are many points of entry whereby stimuli or alterations can change these systems and many points of exit in terms of behavioral responses. In regulation of sleep/wake behavior, every major neurotransmitter system defined in the brain is involved at some level. Therefore, any alteration of activity levels of those neurotransmitter or neuropeptide systems has at least the potential to alter sleep/wake behavior.

Just as there are many neurotransmitter systems involved in the regulation of sleep, many cytokines are now known to be regulators or modulators of arousal state. The two cytokines that have been studied to the greatest extent with respect to their impact on sleep/wake behavior and their role as regulators of sleep are interleukin-1 (IL-1) and tumor necrosis factor (TNF). A variety of other cytokines, chemokines, and growth factors have been implicated at some level as modulators of sleep, but IL-1 and TNF have received the most attention.

Corticotropin-Releasing Hormone and the Regulation of Wakefulness or Arousal

Many studies indicate that, directly or indirectly, corticotropin-releasing hormone (CRH) is involved in the regulation of wakefulness. Studies by Kalin and others demonstrate that there is a circadian rhythm for CRH and that rhythms of CRH and HPA axis activity parallel periods when animals are awake or aroused. Administering CRH to an otherwise normal animal increases electroencephalogram (EEG)-defined wakefulness. When the CRH system is antagonized, EEG-defined wakefulness is reduced; rats deficient in CRH production also exhibit less spontaneous wakefulness. If the system is targeted at the receptor level, the amount of time an otherwise normal animal spends awake can be reduced.

Low doses of CRH administered into the lateral ventricle of rats, either prior to the beginning of the dark portion of the light-dark cycle or prior to the beginning of the light period, increase the amount of time the animals spend awake. The proportional change in the amount of time they spend awake is greater if CRH is administered prior to the light period because this is the time when the animals normally sleep the most.

Lewis and Fischer rats differ in their production of CRH and in HPA axis activity. With Lewis, Fischer, and Sprague-Dawley rats, spontaneous sleep/wake behavior in the absence of any other challenge (except the surgical implantations that allow researchers to determine sleep/wake behavior) was studied. The Lewis rats, which have reduced CRH and which exhibit hypo-responsive HPA axis activity, spend less time awake during both light and dark periods of the light-dark cycle. Therefore, reduced endogenous levels of CRH in an otherwise normal animal are associated with reductions in the amount of time spent awake.

Role of IL-1 in Sleep Regulation

Most cytokines to date have been described initially as products of the immune system. Although 20 years ago the idea that cytokines could be produced within the CNS was viewed with skepticism, now it is known that cytokines and their receptors are found in the brain. They have been implicated in many CNS processes, including arousal state, thermoregulation, appetite and feeding, sexual behavior, social interactions, and mood.

An abundance of evidence indicates that IL-1 is involved in the regulation and modulation of sleep. Starting in the early to mid-1980s, Krueger and colleagues demonstrated that administration of IL-1 into rabbits increases the amount of non-REM (rapid eye movement) sleep. Moldofsky and colleagues demonstrated that concentrations of IL-1 in human plasma peak at sleep onset. In studies done in cats, IL-1-like activity in the cerebrospinal fluid varied in phase with sleep/wake behavior. When IL-1 is given to humans or laboratory animals, the amount of time spent in non-REM sleep increases. Antagonizing the systems reduces the amount of time spent in non-REM sleep. Protein and message expression in the brain demonstrate diurnal rhythms that parallel sleep/wake behavior. If receptors are knocked out and they are not challenged by any other means, laboratory animals spend less time asleep.

One example of how IL-1 affects the sleep of rats is seen in an experiment with Sprague-Dawley rats. A standard dose (5 nanograms) of IL-1 was given through intracerebroventricular (ICV) administration. In the first hour post-injection, there was an increase in the amount of time the animals spent in sleep, but the bulk of increase in non-REM sleep occurred 4 or 5 hours after ICV administration. When IL-1 was given before the dark period, REM sleep was suppressed for 4 to 6 hours. A typical response showed an increase in the amount of time spent in non-REM sleep.

The primary feedback mechanism for cytokine synthesis within the brain is the HPA axis, and it is the action of the glucocorticoids at the level of the hippocampus and hypothalamus that regulate synthesis of IL-1 and TNF.

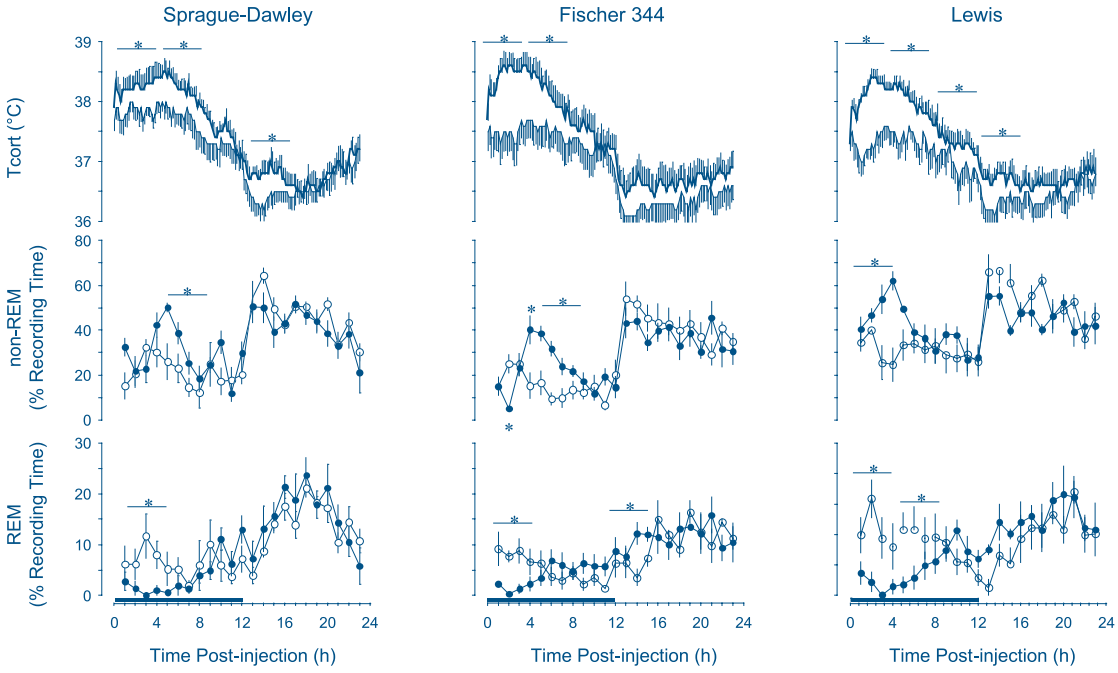
The HPA axis is a critical negative feedback regulatory mechanism for many actions in the brain and for the regulation of immunomodulators, such as cytokines. If animals differ in their HPA axis responsiveness to immune challenge, the precise sleep alterations in response to IL-1 will also differ between these animals. Because Fischer rats are hyper-responsive to immune challenge, and Lewis rats are hyporesponsive with respect to the HPA axis, differential sleep responses are anticipated when each of these strains is injected with IL-1. Fischer rats exhibited a clear difference from Sprague-Dawley rats; specifically, in the first hour post-injection, no increase was seen in the amount of sleep time, and by the second hour post-injection, the animals were awake nearly 100 percent of the time. In comparing the Fischer rat responses to the responses of Lewis rats, it is clear that the time course of response was different. Animals that were hyporesponsive and did not have an exaggerated HPA axis response in the primary negative feedback regulatory mechanisms exhibited a rapid and dramatic increase in the amount of time they spent asleep.

We hypothesized that the second hour pattern of reduced sleep/enhanced wakefulness of Fischer rats after IL-1 was due to a CRH surge. When the animals were pretreated with CRH receptor antagonist before the administration of IL-1, the pattern in the first 2 to 3 hours after IL-1 administration was completely blocked. No reduction in sleep after IL-1 administration was seen when CRH was blocked.

Summarizing these experiments, if an animal is targeted with an immune challenge, there is an impact on HPA axis responsiveness and different types of behavioral outcome measures depending on the direction of the HPA axis response.

If the HPA axis in an otherwise normal animal is targeted, with respect to cytokine systems, will anything change within the CNS or brain? Researchers first targeted the HPA axis in an otherwise normal animal. The primary feedback mechanism for cytokine synthesis within the brain is the HPA axis, and it is the action of the glucocorticoids at the level of the hippocampus and hypothalamus that regulate synthesis of IL-1 and TNF. When the activity of the system is high in a circadian rhythm, as in a rat with the HPA axis activity highest during the dark period, message levels for cytokines in the brain are reduced because of this negative feedback inhibition.

We then investigated what would happen if the HPA axis were antagonized when at its highest level of activity and the impact of glucocorticoids were reduced within the CNS. The hypothesis was that they would see an increase in synthesis in IL-1 at a time when it would normally be reduced. A CRH receptor antagonist (astressin) was administered centrally into rats, which were then sacrificed 4 or 6 hours after administration. Tissues from the brain were processed for message expression for a variety of cytokines, using ribonuclease protection. Samples were taken from the hypothalamus, hippocampus, brain stem, and cortex. It was readily apparent that expression for IL-1 was dramatically increased in the hippocampus, brain stem, and cortex of animals that received the receptor antagonist, compared to those that did not. Quantification of many samples showed changes in both IL-1-α and IL-1-β in areas of the brain that are responsible for the regulation of complex behavior, specifically sleep. We further demonstrated that the effects of intracerebroventricular (ICV) administration of a CRH receptor antagonist on sleep/wake behavior could be blocked completely if the animals were pretreated with an antibody directed against IL-1β.



Effects on cortical brain temperature (Tcort), non-rapid eye movement sleep (non-REM) and rapid eye movement sleep (REMS) of interleukin (IL)-1β injected intracerebroventricularly into rats of three genetically-related strains. Each Sprague-Dawley (n = 7), Fischer 344 (n = 8), and Lewis (n = 7) rat was injected before dark onset with either vehicle (3µl pyrogen-free saline; open symbols, thin lines) or 5.0 ng IL-1β (in 3µl vehicle, closed symbols, thick lines). Values are the hourly means ± SEM. The dark bars on the x-axis indicate the dark portion of the light:dark cycle. * $P < 0.05$ vs. vehicle. From: Opp, M. R. and L. Imeri. *Neuroendocrinology* 73: 273-284, 2001.

Role of IL-6

New data about the role of IL-6 in the regulation of sleep/wake behavior are now available. Evidence is beginning to suggest that IL-6 may at least modulate sleep under some conditions. For example, it is known that in response to IL-1 or TNF, many of the behavioral and physiological outcome measures are actually mediated by IL-6. Bauer demonstrated some years ago that there is a rhythmic activity to IL-6 concentrations in human subjects. When human volunteers are sleep-deprived, IL-6 increases. If IL-6 is injected into human volunteers, the amount of time they spend asleep increases during the latter portions of the night. We recently reported that when IL-6 was administered ICV into rats, there were periods of the recording time during which non-REM sleep increased. It has been known for some time that IL-6 is a powerful stimulator of HPA axis activity. Therefore, there are many reasons for interest in the sleep of IL-6 knockout mice.

One study compared the 24-hour pattern of body temperature and non-REM and REM sleep in C57BL/6 mice and IL-6 knockout mice. The circadian rhythm of body temperature is low during the rest period, or the light period of the light-dark cycle, and higher at night when the animal is active. This was evident and the amount of time spent in non-REM sleep and REM sleep was charted. The compared data show that there was no difference in timing of sleep in the two strains, or in the rhythms of body temperature. However, IL-6 knockout mice spent more time in REM sleep than did the C57BL/6 mice. The amount of time an animal spends asleep is one measure of sleep/wake behavior. Another measure is the EEG, which shows characteristic patterns and frequencies during the different stages of sleep. In spectral analyses of EEGs from periods of non-REM or REM sleep or wakefulness, the characteristic spectra did not differ between the C57BL/6 and IL-6 knockout mice.

We then looked at how the mice responded to a homeostatic challenge, specifically to periods of sleep deprivation. If the C57BL/6 mice were sleep deprived for 6 hours at the beginning of the light period and then allowed to sleep normally after that deprivation, rapid increases in the amount of time they spend in non-REM and in REM sleep occurred. IL-6 knockout mice subjected to 6 hours of sleep deprivation showed a similar pattern; sleep deprivation affected their REM sleep in the same manner as C57BL/6 mice. The effect of sleep deprivation on non-REM sleep differed in that the IL-6 knockout mice took longer to recover after the deprivation period ended. The EEG correlates after sleep deprivation show that the increase in intensity measures of sleep (the delta power) was identical in C57BL/6 and IL-6 knockout mice. The IL-6 knockout mice slept normally when they were not challenged, and they reacted similarly in response to a moderate homeostatic challenge, such as 6 hours of sleep deprivation. Lack of a functional IL-6 gene did not seem to greatly affect any of the parameters studied.

Of greater interest is how the mice respond to an immune challenge. After intraperitoneal administration of 10 micrograms of bacterial lipopolysaccharide (LPS), C57BL/6 mice initially experienced a brief period of hypothermia. A febrile response then developed about 6 to 8 hours after injection, with a dramatic increase in the amount of time spent in non-REM sleep. This occurred relatively quickly, and the suppression of REM sleep was finished within 12 hours. In contrast, IL-6 knockout mice showed a significant hypothermic response. Temperatures went down 5 to 6 degrees, then recovered, and were back to baseline within 12 hours. Enhancement of non-REM sleep of IL-6 knockout mice in response to LPS was exactly 50 percent of the enhancement observed in C57BL/6 mice, and suppression of REM sleep was virtually identical to that of the C57BL/6.

After administration of LPS, both strains, when asleep, exhibited a reduction in EEG delta power—the frequency band that is primarily manifest during non-REM or slow-wave sleep. However, by 5 to 6 hours after injection, the C57BL/6 mice were exhibiting slightly more power in the delta frequency band, whereas the IL-6 knockouts remained reduced in this measure. Focusing specifically on the one hour when the difference between the two strains was greatest, it was apparent that there was an increase in this intensity measure in C57BL/6 and a reduction in the IL-6 knockout mice that was characteristic of all the frequencies analyzed in the study. The IL-6 knockout mice responded much differently to immune challenge than did the C57BL/6 mice, even though other aspects of sleep/wake behavior were similar under nonchallenged conditions or in response to a relatively modest homeostatic challenge. These changes could be detected in the animals, both at the level of gross behavior and at the level of brain activity, as reflected in EEG patterns.

Conclusion

As these studies demonstrate, CRH and the HPA axis are generally involved in the modulation of arousal. Most pro-inflammatory cytokines that have been studied to date, at some point and under some conditions, will increase the amount of time animals spend in non-REM sleep. Interactions between many different neurotransmitters, but certainly between CRH and the HPA axis and cytokines, are of functional consequence to behavioral outcome measures. Any stimulus that alters the normal expression of these systems has the potential to alter sleep/wake behavior and contribute to excessive daytime sleepiness or disrupted nighttime sleep.

Discussion

Dr. Sternberg: Is there a difference in the males and females in this case? Is there a critical age period?

Dr. Opp: There are definitely gender- and age-related differences. We have not looked at female IL-6 knockout mice. We have conducted some aging studies using the Fischer rat, which are in the process of being analyzed. We have not looked at any gender differences.

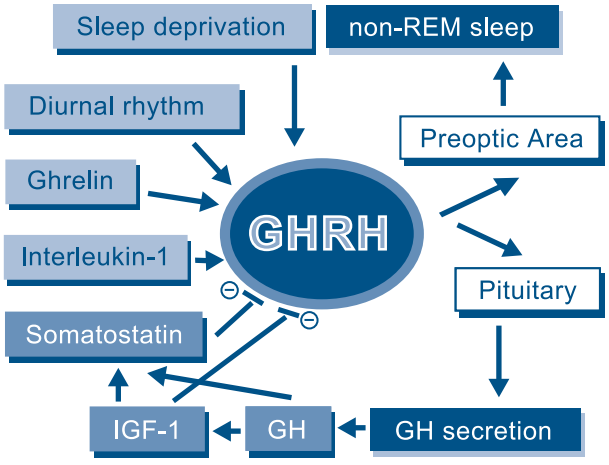
Dr. López: When you gave the CRH antagonist, you saw an increase in IL-1, and you also said that the glucocorticoids decrease the expression of interleukins in these regions. Would you expect giving CRH to abolish the effect in non-REM sleep in these animals?

Dr. Opp: It is a question of timing. These two systems are inversely related to each other with respect to their expression. We antagonized the system during the dark period when HPA axis activity is high and the message would normally be low for IL-1. By breaking that negative feedback, in addition, releasing that inhibitory tone, the message was increased. Had we done the same manipulation 12 hours later, I am convinced we would not have seen that result, because these systems must be antagonized at the appropriate time. If you increase the activity of the HPA axis system, then you will certainly affect cytokine- or IL-1-mediated behaviors in the brain.

Mechanisms Underlying the Central Effects of Cytokines

James Krueger, Ph.D.

Sleep is regulated, in part, by a brain biochemical network. During the past 20 years, many components of this network have been identified and the specific links between a sleep regulatory substance and behavior have been characterized. This presentation focused on three of these sleep regulatory substances: growth hormone-releasing hormone (GHRH), interleukin-1-β (IL-1-β), and tumor necrosis factor-α (TNF-α). The characterization of these substances as sleep regulatory substances grew out of an earlier literature which demonstrated that the transfer of cerebrospinal fluid from a sleep-deprived animal to a normal animal enhanced sleep in the recipient. Further characterization of the involvement of GHRH, IL-1, and TNF in sleep regulation followed, in part, a classic experimental paradigm developed in endocrinology—that of using pathology to identify physiological mechanisms. Thus, in one or more disease states GHRH, IL-1, and TNF are altered and those changes are associated with sleep disturbances. It seems likely that the fatigue and sleepiness associated with chronic fatigue, regardless of etiology, are associated with changes in these molecules.



Growth Hormone-Releasing Hormone

Linkage exists between pituitary release of growth hormone and sleep. Takahashi in 1968 showed that about 50 percent of daily growth hormone output in humans occurs in the first non-REM sleep period; this solid finding in humans has now been extended to other animals. On the other hand, situations in which plasma levels of growth hormone could be separated from sleep regulation were also known. In the mid-1980s, it was hypothesized that GHRH could induce non-REM sleep and it can also induce growth hormone release, but those mechanisms are anatomically separate.

When GHRH was given to rabbits, the highest doses provided the longest effects in promoting non-REM sleep. Microinjecting into the intrapreoptic area of the hypothalamus induced these increases. There was no effect on temperature. These doses also induced increases in REM sleep. When the animals' pituitary glands were removed, only the increases in non-REM sleep occurred, indicating that the effects on REM sleep are mediated by growth hormone while non-REM sleep effects of GHRH are mediated through GHRH itself.

Intrapreoptic injection of a peptide antagonist of GHRH inhibited spontaneous sleep, suggesting that GHRH is involved in spontaneous sleep. Feedback mechanisms related to internal GHRH and GH production involve somatostatin and insulin-like growth factor (IGF)-1, both of which are negative feedback signals. After administration of octreotide (a long-lasting somatostatin analog) or IGF-1, there is an inhibition of growth hormone release and non-REM sleep, most likely because it is feeding back and turning off GHRH release. GHRH release also can be inhibited in other ways, for example, with antibodies.

GHRH message production and release of GHRH from the hypothalamus co-vary with non-REM sleep. This is corollary evidence only. During periods of sleep deprivation, synthesis of GHRH goes up and GHRH synthesis release is correlated with non-REM sleep.

In one experiment injecting GHRH, we used the lit/lit mouse, a model with a GHRH receptor deficiency caused by a single base mutation that results in a single amino acid substitution in the protein for the growth hormone-releasing receptor. Called lit/lit because they are little, these mice are small because the GHRH receptors are nonfunctional; they do not recognize GHRH and therefore do not release pituitary growth hormone. When the control animals were given GHRH, their amount of sleep increased. The lit/lit mice did not respond to GHRH and had substantially less non-REM and less REM sleep than the controls. With growth hormone therapy, lit/lit mice started growing and their IGF-1 increased. Non-REM sleep was unchanged but REM sleep returned to normal, indicating that the effects of GHRH on REM sleep are mediated via the growth hormone.

Cytokines

It has been known for some time that when a person is ill, growth hormone levels go up. At least 15 years ago, studies determined that IL-1 stimulates growth hormones. In one rat study, 2.5 nanograms of IL-1 were injected, leading to increases in growth hormone. It was clear that the increases were GHRH-mediated because animals pretreated with GHRH antibody and given IL-1 did not demonstrate similar increases in growth hormone. In simultaneous sleep experiments, non-REM sleep increased in rats given IL-1. In animals pretreated with GHRH antibodies, there was less spontaneous sleep and virtually no response to IL-1, indicating that IL-1-induced non-REM sleep involves GHRH.

With current technological advances that afford closer looks at cellular mechanisms, we began to examine the responses of cultured hypothalamic neurons to IL-1-β and GHRH to see if there was a co-localization of the receptors on the same neurons. Measuring cytosol calcium levels, intracellular calcium increased with IL-1; the same thing happened when GHRH was administered. The cells are now known to be receptive for both GHRH and IL-1; there is a co-localization. These cells are also known to be GABA-nergic, which dovetails with the sleep literature.

Demonstrating a further relationship between IL-1 and GHRH, when IL-1 is given *in vitro*, it induces production of the GHRH receptor. Not only do the receptors co-localize in the same cells in the hypothalamus, IL-1 can induce GHRH receptor, although it is not clear whether the animal is more susceptible to GHRH after receiving IL-1. While it has been shown that IL-1 can work to induce sleep via GHRH, it is not known if the reverse is true.

In a study in our laboratory, we infected lit/lit and control mice with influenza. The control had a normal response to influenza, sleeping more with somewhat flattened rhythms, but the lit/lit mice slept less, with substantially less survivability. This suggests that the GHRH receptor is an integral part of the response to influenza challenge, in terms of sleep and survivability. Administrating growth hormone did not rescue the animals, indicating a true GHRH receptor-mediated event that is not GH dependent. When IL-1 was administered to control and IL-1 receptor knockout mice, the responses of the knockout mice demonstrated that the receptor is involved in the sleep response to IL-1.

IL-1 is related to sleep and it is in the brain. Examples from the hypothalamus, hippocampus, and cortex showed spontaneous levels of IL-1 in a regular diurnal rhythm in all three areas of the brain. The message levels were higher during the day, when the animals were sleeping, than at night. In sleep-deprived animals, cytokine message levels went up in the four areas of the brain measured: hippocampus, hypothalamus, brain stem, and cortex. Protein levels also increased. These effects were specific to IL-1-β.

These findings shed light on why, for example, a lung disease such as influenza induces brain symptoms even though the disease is largely confined to the lungs.

An experiment by one of my graduate students indicated at least one mechanism by which cytokines signal the brain. In the context of an experiment testing how feeding affects sleep, rats were given a junk-food diet. They ate more than on a normal diet and slept more, and the effects were shown to be IL-1 dependent. Animals were injected intraperitoneally with IL-1. The IL-1 that was signaling the brain was probably produced peripherally. IL-1 induced its own messenger, a finding that has been replicated in other work. Vagotomy on the animals

did not affect IL-1 production in the liver because nothing was blocked; there was still an increase in IL-1 messenger. In the brain stem, however, the induction of IL-1-β was completely blocked. It is known that the vagus nerve contains receptors for IL-1. IL-1 was injected and it bound to receptors on the vagal nerves; action potentials were elicited and the action potentials made their way to the brain and were somehow translated into increased messenger production for IL-1. This can be blocked with a vagotomy.

Cytokines are known to induce fatigue. Whether this fatigue is the same as what is seen with CFS is not known.

These findings shed light on why, for example, a lung disease such as influenza induces brain symptoms even though the disease is largely confined to the lungs. Many of the brain symptoms occur before the edema that accompanies influenza; these effects are dose dependent and are not seen with higher doses, which can get in the brain by other mechanisms. They can be transported across the blood-brain barrier, they can go through leaky areas, and they can induce secondary molecules, such as prostaglandins, and then make their way through the blood-brain barrier.

The cytokines referred to in human studies are circulating cytokines, whereas in animal work central production of cytokines can be studied. This creates two bodies of evidence, one looking at circulating levels and the other looking at central levels; when they yield differing answers, it may be necessary to determine what the circulating cytokines do. Considerable evidence points to the ability of circulating cytokines to signal the brain through a variety of mechanisms, including the vagus nerve. Cytokines are known to induce fatigue, as is documented in some studies of humans; an example is patients who report fatigue and sleeping more when injected with IL-1. Whether this fatigue is the same as what is seen with CFS is not known.

The biologic effect of cytokines is dose dependent. Low doses tend to have positive results; for example, low doses of TNF are neuroprotective against ischemic damage to the brain and high doses do the opposite. Low doses of IL-1 and TNF promote sleep; high doses do the opposite, due to feedback mechanisms. Cytokine doses are extremely small, so these are potent substances.

Tumor Necrosis Factor

TNF messenger RNA and protein levels vary in phase with the sleep/wake cycle in a pattern that is correlated but not proven to be causative. Injecting TNF produced causative data. In sleep-deprived rats there was an upregulation of TNF messenger RNA in the hypothalamus. Microinjection of TNF into the preoptic area led to increases in non-REM sleep but had little effect on REM sleep. When an inhibitor was microinjected—in this case the TNF soluble receptors, which increase in human plasma in times of sleep deprivation and are a normal component of cerebrospinal fluid in humans—spontaneous sleep was inhibited. This fits well with the sleep literature, which indicates that the preoptic area is used for sleep regulation.

There is some evidence that TNF is produced in response to action potentials in neurons. Much of this data comes from researchers interested in kindling, a process of development of epilepsy in animal subjects. Increases in brain production of TNF are seen in the process. We have examined *in vitro* neurons after they have been treated with a glutamate stimulus. A dose-dependent increase in TNF production was seen, both in the media and in the cells.

Some data suggest that TNF is released as a function of its own use. Sleep is targeted to areas of the brain depending on prior neuronal use; how much a person has used his or her brain depends on how much the brain sleeps or its level of sleep intensity.

A hypothesis regarding sleep intensity is that the unilateral application of sleep regulatory substance should induce EEG asymmetries. In earlier studies of factor S, a sleep regulatory substance, high-amplitude slow waves were seen that initially looked like an artifact; however, sleep deprivation, which endogenously built up sleep regulatory substances, also showed the high-amplitude slow waves. This provided a measure of intensity of sleep, and there is now a large body of literature supporting this concept.

If TNF is put on the surface of the cortex just under the dura, a difference between the two sides is evident: the amplitudes are higher on one side of the brain. In time-dependent and frequency-dependent fashion, low doses induce an increase in amplitude in slow-wave activity. Higher doses induce larger effects that are more persistent. These results mimic the consequences of sleep deprivation. When there is sleep

rebound, if the sleep deprivation was short, the rebound is usually confined to non-REM sleep with little effect on REM sleep.

Several laboratories have shown that TNF in the brain signals through the AMPA receptors, and AMPA receptors are needed for EEG synchronization. The downstream events of the cytokines IL-1, TNF, and GHRH have been well described. Nitric oxide is a common downstream event for many of the substances that are known to affect sleep.

Studies of knockout mice in my laboratory determined spontaneous sleep in two different types of mice lacking either neural nitric oxide synthase (nNOS) or inducible nitric oxide synthase (iNOS). The major finding was that if the animals lacked nNOS, their non-REM sleep was normal and their REM sleep was substantially reduced, especially during daylight hours. In contrast, if the animals lacked iNOS, they had more REM sleep than normal and less non-REM sleep. These studies and a variety of others indicate that nitric oxide and its enzymes that control synthesis, nitric oxide synthase, are involved as a downstream event, at least for effects on REM. That conclusion is generally accepted, but the role of nNOS or iNOS in non-REM sleep is more controversial.

Theoretical Considerations

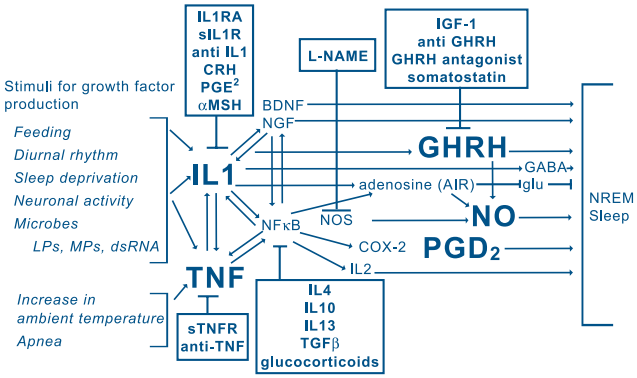
Thinking about biochemical sleep mechanisms leads to a new way of thinking about how the brain is organized to produce sleep and what sleep evolved to do. This knowledge is related to CFS because part of the brain can be asleep while another part of the brain is awake, which eventually might have implications for CFS treatment and understanding the syndrome.

A neuronal group theory of sleep function was published about 10 years ago. The following are the main tenets of that theory:

- Sleep is a fundamental property of groups of highly interconnected neurons, called neuronal groups. Other researchers have hypothesized that there are more than 100,000 of these groups within the brain. An example of what these groups look like is the individual barrels of the sensory cortex of a rat, where about 10,000 neurons are organized around a single facial whisker, which receives afferent input and then sends signals to other parts of the brain.
- Homeostatic sleep mechanisms cannot be separated from sleep function. The molecules that are known to be involved in sleep regulation provide information about sleep function.
- Sleep function deals with neural connectivity. This is not to suggest that sleep serves a single function any more than the lungs serve a single function. Sleep probably evolved for a primordial function and, as it was evolving, other functions evolved with it. That primordial function deals with neural connectivity.
- The need for sleep is derived from the advantages of a flexible microcircuitry. Living organisms could have evolved as fixed, hard-wired computers or with flexible wiring of the brain; the advantages of flexible wiring are immense.

A discussion of sleep mechanisms expands upon these ideas. First, there is an activity-dependent production of sleep-regulatory substances. Neurons produce substances, dependent upon how active those neurons are. The concept that neurons produce substances in response to changes in membrane potential across the extracellular membrane is not new, but researchers are now seeing that those changes lead to the production of sleep-regulatory substances. One sleep-regulatory substance is nerve growth factor and another is brain-derived neurotropic factor. Both of these molecules are released from neurons as a function of neuronal activity. The release of other sleep-regulatory substances is also dependent on past neuronal activity.

The activity-dependent, sleep regulatory substances act in autocrine, juxtacrine, and paracrine fashions to change the electrical properties of nearby neurons and thereby alter input/output relationships. A large body of literature demonstrates that many different compounds, when applied onto a neuronal network, will change the input/output relationships of that network. One of the better studies of interest to fever researchers is IL-1 put into hypothalamic networks; researchers then study the effects on the hypothalamic neurons, including their sensitivity to temperature. IL-1 changes the sensitivity; for the same input, there is a different output.



The altered input/output relationship for a neuronal group is, by definition, an altered state. For example, where an environmental input gives an output that makes sense in terms of the environment this can be characterized as an awake state or a deterministic state. In contrast, if that input/output relationship shifts, at some point the output will not make sense out of the environmental input (a chaotic state).

Sleep is not only *for* neural connectivity, but also *because* of it.

The activity-dependent sleep regulatory substances are also growth factors. They provide for the structural basis of synaptic efficacy and neural connectivity. This is not controversial at all for some of the molecules known to be sleep regulatory substances, for example, nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and nitric oxide. Others (for example, IL-1 and TNF) are more controversial in terms of their roles in brain connectivity but may be less so as time goes on.

The altered input/output relationships provide stimulation for, and thereby preservation of, synapses not stimulated by the prior environmental input. These alterations stimulate the synapses; therefore, the efficacy of transmission of action potentials or electrical signals through the synapses is enhanced. Expressed in plasticity modeling terms, Hebbian processes dominate during waking while scaling processes characterize sleep.

A number of substances have been well characterized as sleep-regulatory substances and are known to be involved in some aspect of synaptic plasticity or neural connectivity. These include:

- *Non-REMS*: GHRH, TNF, IL-1, adenosine, PGD2, NGF, BDNF
- *REMS*: VIP, PRL, NO, Ach
- *W*: CRH, hypocretin, NA 5-HT, Ach

These substances are characterized as Process S, one of two branches of a two-process model of sleep regulation developed by Borbély. Process S is a homeostatic process and consists of buildup of sleep-regulatory substances during wakefulness and dissipation during sleep. The other part of the model is a circadian process, Process C, which sets the levels at which sleep occurs and does not occur.

Sleep-regulatory substances that have been demonstrated to have activity-dependent production are NGF, BDNF, IL-1, TNF, nitric oxide, adenosine, and neural transmitters. For all of these substances, it is known that there is an activity-dependent production and release; this is how the brain can target sleep to areas dependent on past activity.

A long list of sleep regulatory substances are implicated in synaptic plasticity: NGF, BDNF, IL-1, NO, PRL, VIP, PACAP, adenosine, prostaglandins, somatotropic axis, EGF, FGF, GDNF, NT3, NT4, IL-2, IL-6, IL-8, IL-18, IFN-α, TGF-β, PAF, and estrogen. Not all are well characterized as sleep-regulatory substances, but all have been shown in at least one laboratory to affect sleep. Most of them promote sleep, but some actually inhibit sleep, such as TGF-β. This reinforces the view that sleep has something to do with plasticity, since most of the substances that affect sleep also affect plasticity.

Sleep is not only *for* neural connectivity, but also *because* of it. The sleep need is derived from the flexible microcircuitry, its use-dependent rules, and the necessity of preserving those synaptic networks responsible for innate and learned memories. Some experimental evidence suggests that sleep is involved in neural connectivity. The bigger question about the need for sleep and where that need comes from is critically important, because the answer to this question will provide insights into better clinical care in conditions such as CFS.

In rat studies, we have determined that sleep loss and sleep gain affect the expression of molecules linked to synaptic plasticity. Levels of mRNA after sleep deprivation and excess sleep (triggered by increased ambient temperature) were examined in three molecules. The first was BDNF, which is not only known to be involved in plasticity but is also a sleep-regulatory substance. The second was activity-related cytoskeletal (ARC) protein, which is one of the better-characterized molecules within the plasticity field. Its messenger RNA is found in the dendrites and it is expressed as a function of local activation or local changes in excitatory post-synaptic potentials within the dendritic tree. This allows targeting not only an area of the brain, but also what is in a cell for induction of ARC message and translation of the message into protein. The third substance is matrix metalloproteinase 9 (MMP-9), also called gelatinase B. It is also involved in plasticity, although its involvement is, in one sense, the opposite of ARC: it digests the extracellular matrix to make a new synapse.

In all of these compounds, the sleep effect is evident in the expression of their mRNAs. BDNF and ARC increased with sleep loss whereas MMP-9 decreased. When the animals slept more, mRNA went in the opposite direction: BDNF and ARC decreased and MMP-9 increased.

Well-characterized plasticity molecules seem to be affected by sleep. However, there are thousands of molecules involved in plasticity and not every one of them will prove to be sleep sensitive. This is a complex field, and it will take many years to work out the relationship between sleep and plasticity but this is a beginning, and other researchers are beginning to direct their efforts to this area. Research on NGF immunoreactivity by graduate students in our laboratory tested the effects of sleep deprivation, which alone resulted in increases in NGF immunoreactivity. The number of cells increased and the amount within individual cells also increased, and NGF distribution within the cell changed. The next part of the experiment used the rat whisker cut model, which is popular in the field of plasticity. Researchers cut the whiskers on one side of the face, which reduced afferent activity into the contralateral somatosensory cortex because the whiskers did not get stimulated as much. The whisker cut was then combined with sleep deprivation. The brain on one side, with intact whiskers, was relatively normal with appropriate input, but the side linked to the cut whiskers had much less input.

On the intact side, sleep deprivation enhanced NGF immunoactivity. However, on the side that had reduced activity because of the cut whiskers, instead of enhancing expression of NGF, sleep deprivation reduced its expression. This clearly showed an interaction between sleep loss and the ongoing changes that occur within a neuronal group. If the whiskers are cut on one side of the face, within 24 hours the neurons will begin to reorganize their connectivity on the opposite side of the animal. They will start to connect with other neurons from other neuronal groups or other barrels that are involved in afferent input from different (uncut) whiskers. Sleep appeared to affect that reorganization, or at least affect molecules involved in plasticity, in a different way than if the whiskers were intact.

Theoretical constructs currently under consideration and testing will, over time, tell a great deal about how the brain is organized to produce sleep, what sleep is about, and provide new avenues to change how the brain operates, allowing scientists to target parts of the brain during wakefulness or during sleep.

Session Four: Will Understanding Central Mechanisms Enhance the Search for the Causes, Consequences, and Treatment of CFS?

Chair: Dedra Buchwald, M.D.

This session of the conference holds implications for designing studies and answering questions about the nature and causes of chronic fatigue syndrome, as well as the existence of neuroimmune modulators or influences. New methodologies include innovative study designs, such as twin and family studies, studies of other disorders, use of new technologies or new applications of existing technologies, and thinking about CFS in a different way or using a different approach. For example, rather than investigating symptoms or problems that CFS patients develop, perhaps questions should be asked about the symptoms and problems not developed by CFS patients and how that knowledge might inform the field about the pathophysiology and mechanisms behind CFS.

Brain Stress–Response Circuitry in the Regulation of Physical and Affective States

Jon-Kar Zubieta, M.D., Ph.D.

A multiplicity of symptoms characterize chronic fatigue syndrome (CFS). Acute onset and persistence of fatigue is the defining symptom, but other symptoms are part of the CFS diagnosis, including (in order of frequency) sleep disturbances, muscle weakness, difficulty concentrating, worsening with stress, joint and muscle pain, difficulties with attention and concentration, weight gain, increased need for sleep, and headaches. The causes of CFS are unknown, but its manifestations are multiple and include both body (such as muscle fatigue and pain) and brain (central nervous system) symptoms. More women than men are diagnosed with CFS. In a manner similar to other physical illnesses, such as chronic pain or other physical disabilities, its permanence and continuous impact on the life of those affected can induce complicating syndromes, such as major depression, itself an illness typically induced by emotional and physical stress.

Other idiopathic forms of persistent pain which complicate CFS, such as fibromyalgia syndrome, temporomandibular joint pain, and major depression, are also more frequently diagnosed in women of reproductive age. CFS is a more common diagnosis in monozygotic than in dizygotic twins, suggesting that some vulnerability for the initiation or permanence of CFS may be due to genetic influences. Genetic, gender-related, and stress-related factors, therefore, should be examined for their contribution to the pathophysiology and manifestations of CFS. (Stress is defined broadly here as insults—physical, such as chronic illness or pain, or emotional, such as severe life events—that destabilize the homeostasis of the organism.)

We are therefore describing a research avenue that necessitates a systems-level understanding of this illness and an illness that falls into complex pathologies in which genetic, psychophysical, and environmental factors interact. In that regard, CFS is not unique, and it joins syndromes as diverse and as poorly understood as persistent muscular pain, dysautonomias, depression, and irritable bowel syndrome. This perspective has relevance for the types of approaches needed to unravel the multiple manifestations of CFS. Complex illnesses are not characterized by a single phenomenon that leads to an illness process; they are caused by interactions between biological or genetic vulnerabilities and other factors, such as infections, injury, and environmental factors that may foster the transition from vulnerability to a pathological state. For example, major depression, another illness of complex etiology, can develop in vulnerable individuals as a result of interactions among genetic vulnerability and factors, such as life events, stressors, infections, injuries, or persistent pain, and it is more frequently diagnosed in women. Gender, hormones, or both may then induce additionally increased predisposition toward certain complex illnesses in which a gender predominance is observed, such as in CFS or persistent pain syndromes.

We are therefore describing a research avenue that necessitates a systems-level understanding of this illness and an illness that falls into complex pathologies in which genetic, psychophysical, and environmental factors interact.

My discussion centers on possible perpetuating mechanisms that aggravate the course of CFS and may induce a vicious circle of vulnerability to dysregulation to further worsening, focusing on the CNS, a locus of manifestation of a number of CFS symptoms. I review the literature on central nervous system findings in CFS, and recent studies from our and other laboratories that exemplify neurobiological models of interaction among genes, gender, stress, pain, and affective regulation as possible avenues for discovery in CFS.

Initial studies with magnetic resonance imaging (MRI) in patients suffering from CFS have shown areas of increased signal intensity, typically interpreted as reflecting neuronal changes due to edema or demyelination.[1,2] Changes in the blood flow and the glucose metabolism of various brain regions, primarily frontal and temporal, have also been reported during baseline, neutral states in CFS.[3-6] However, these findings have not been replicated in other studies, notably one comparing monozygotic twins discordant for the presence of CFS, in which genetically related variations in brain metabolism are controlled for in the design.[7] In addition, none of the positive findings with these baseline measurements have demonstrated changes during resting states that are defining or specific for CFS. Measures of regional cerebral blood flow, metabolism and, more recently, functional MRI responses to various stimuli are related to synaptic activity during the study conditions. Baseline measurements most likely reflect the CNS representation of symptoms of the illness, which in isolation are again not unique for CFS.

The question then remains as to what circuits and mechanisms in the brain may participate in the interaction between possible genetic vulnerabilities, stress, and gender to contribute to the manifestations of CFS. None of us has the answer to that question. However, for the purposes of exploring new avenues of investigation, I will draw some parallels from recently acquired data in experimentally induced sustained pain using imaging techniques to more specifically examine the neurochemical systems involved in its regulation in humans. We utilized positron emission tomography (PET) with radiotracers that specifically label receptor systems, affording an additional level of neurochemical specificity that cannot be obtained with measures of metabolic or synaptic activity.

As experimental pain goes from acute (lasting from a few seconds to a few minutes) to more sustained (lasting more than 5 or 10 minutes), its sensory qualities, which define location, perceptual quality, and intensity, remain constant; its affective qualities, which relate to how unpleasant and emotionally burdensome it becomes, sharply increase.[8] Pain is then transformed from an organism's warning signal to a stressor that threatens well-being and activates the production of stress hormones, such as cortisol. It is therefore not surprising that painful and noxious stimuli have frequently been utilized in animal models as a model of stress, in a manner similar to other stressors such as those posed by environmentally adverse events.[9-12]

The question then remains as to what circuits and mechanisms in the brain may participate in the interaction between possible genetic vulnerabilities, stress, and gender to contribute to the manifestations of CFS.

What happens in human subjects as pain becomes more sustained and therefore becomes a stressor? Aside from the initial transmission of pain signals to brain regions involved in its representation,[13] sustained pain activates mechanisms that attempt to suppress it, so some degree of homeostasis can be re-established. We have studied the latter mechanisms directly in humans with PET and radiotracers labeling receptors involved in the suppression of stress and pain. One of these receptors is the μ-opioid receptor. These receptors mediate some of the actions of endogenous opioids—peptide neurotransmitters that are released in response to various stressors, including pain and inflammatory processes. The μ-opioid receptors are also the targets of opiate medications used to reduce acute or chronic pain.

We have utilized a model of sustained muscular pain induced by the introduction of small amounts of hypertonic saline into the jaw muscle to temporarily induce temporomandibular pain, a frequently observed complicating symptom in CFS by itself and together with fibromyalgia. The pain is maintained constant at low levels: 40 units of intensity as graded from 0 (no pain) to 100 (most pain imaginable) as rated by the subjects. Obtained every 15 seconds with the aid of an electronic visual analog scale, pain ratings are fed back into a computer controller that modifies the infusion rate of the hypertonic saline infusion, so that pain is maintained as constant as possible for the duration of the challenge (typically 20 to 30 minutes). This stimulus activates the hypothalamic-pituitary-adrenal hormonal stress responses, inducing increases in the production of ACTH and cortisol.[14] Simultaneously, subjects were scanned with PET and the radiotracer [^{11}C]carfentanil, which selectively labels μ-opioid receptors, during pain and during a nonpainful challenge (isotonic saline) infused in the same manner as the noxious stimulus.

In an initial series of studies in 20 healthy volunteers (14 men and 6 women, 20 to 30 years of age), we demonstrated that the pain stimulus was associated

with activation of endogenous opioid neurotransmission, observed experimentally as reductions in the binding measure obtained with PET, [^{11}C]carfentanil, and kinetic quantification models. As the endogenous neurotransmitter is activated, it competes with the radiolabeled tracer; less μ-opioid receptors are available for the radiotracer to bind, hence the reductions in the *in vivo* binding measure. Significant activation of μ-opioid receptor-mediated neurotransmission was observed in a number of brain regions, including the prefrontal, anterior cingulate, and insular cortices; thalamus, both anterior and lateral; hypothalamus; and in the ventral basal ganglia (nucleus accumbens and ventral pallidum), amygdala, and periaqueductal gray.[15] Retrospectively, and given the widespread distribution of μ-opioid receptors in the human brain, the large number of regions involved in these responses seem reasonable, since pain is a complex experience; however, this result had not been observed previously in animal models. The regions involved are associated with complex processes, including attention and cognition-emotion integration (prefrontal cortex, anterior cingulate); bodily sensory perception and regulation (thalamus, insular cortex); hormonal responses (both stress related, such as ACTH production, and reproductive, hypothalamus); reward and salience evaluation (nucleus accumbens, ventral pallidum); assignment and regulation of emotional valence and stimulus intensity (amygdala); and pain control (periaqueductal gray).

These results emphasized the important role of the CNS in the perception and regulation of stressful stimuli (in this case sustained pain stimuli) and how a neurotransmitter system, the endogenous opioid, and μ-opioid receptors, thought to be primarily involved in analgesia, also affect regions involved in various elements of the stress response, including higher order, attentional, and cognitive functions. Perhaps more importantly, we observed a wide range of interindividual variations in baseline binding, as well as in the capacity to activate μ-opioid receptor-mediated neurotransmission, even though the pain model was controlled for intensity. The magnitude of activation of μ-opioid receptors, calculated as the percent reduction in binding from control to pain scans, was further correlated with ratings of the sensory quality of the pain, its affective ratings, and the negative affective state induced by the noxious stimulus, albeit in distinct neuroanatomical localizations.[15-16] In other words, the capacity to suppress various elements of the pain-stress experience was highly variable among individuals. Subsequent work was then oriented toward understanding the sources of this interindividual variability.

Pain stimulus was associated with activation of endogenous opioid neurotransmission.

We also examined whether men and women differed in their capacity to activate this pain/stress suppressive neurotransmitter system.[16] The gonadal steroid status in the women was controlled by performing the studies in the early follicular phase of the menstrual cycle, when estradiol and progesterone are lowest. Binding measures were obtained under no-pain and pain conditions in samples of 14 each, healthy young (20 to 30 years of age) men and women. Significant differences in the magnitude of activation of the endogenous opioid system and μ-opioid receptors were observed. Consistently, men activated μ-opioid neurotransmission more efficiently than women did, reaching statistical significance in the thalamus, nucleus accumbens, and ventral pallidum and amygdala. This was associated with a more efficient suppression of the ratings of pain in terms of its sensory qualities (nucleus accumbens, amygdala), affective quality of the pain (anterior thalamus), and the negative affective state induced by the challenge (ventral pallidum). One more observation arising from these results was that, in women, substantial interindividual variation was still observed that was not related to plasma levels of estradiol or progesterone, with 4 out of 14 women showing responses that were more similar to those of men. We have further studied these variations with two different approaches: by varying the plasma levels of estradiol and progesterone (work currently in progress) and by examining genetic variations that affect some of the circuitry involved.

In an initial approach to examine the contribution of genetic polymorphisms to the regulation of pain stress, we focused on a common genotype variant affecting the function of the enzyme catecholamine-ortho-methyl-transferase (COMT).[17] Substitution of valine (val) by methionine (met) at codon 158 of the COMT gene induces a reduction in the activity of the enzyme. This single nucleotide polymorphism is codominant, meaning that individuals with two copies of the met allele, approximately 20 percent of the population,[18] have the lowest COMT function, those with one of each have intermediate function

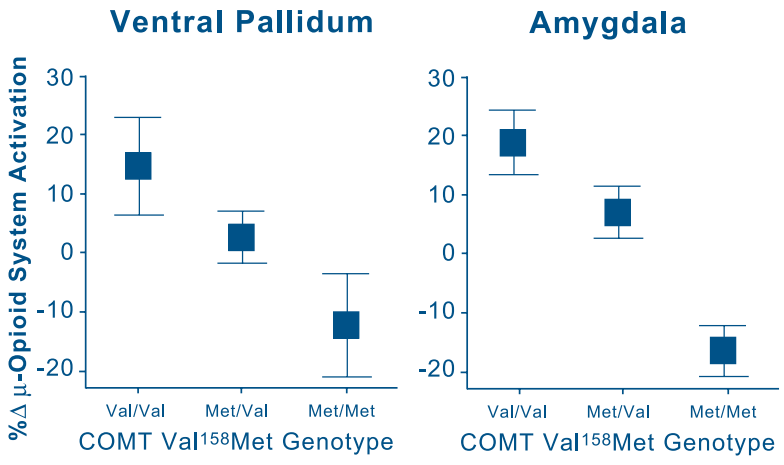
(about 50 percent of the population), and those with two copies of the val allele have the highest function (about 30 percent of individuals).

Our interest in the COMT enzyme arose from its role in the metabolization of dopamine and norepinephrine, both neurotransmitter systems involved in the regulation of stress and responses to salient stimuli. Dopamine is an abundant neurotransmitter in some areas in which large interindividual variations in the response of the opioid system were observed, such as the ventral basal ganglia (nucleus accumbens and ventral pallidum), as well as in the rostral anterior prefrontal cortex, anterior cingulate, and amygdala. At the level of the basal ganglia, studies in animal models had shown that the persistent activation of dopaminergic neurotransmission (a situation presumably similar to that of a low-function COMT enzyme, with lower capacity to inactivate dopamine) reduced the neuronal levels of endogenous opioids and induced compensatory increases in baseline μ-opioid receptor binding.

We then tested whether variations in the function of COMT due to this polymorphism would be associated with variations in the human capacity to suppress the pain–stress experience through the activation of endogenous opioid neurotransmission on μ-opioid receptors. Conducted in 29 healthy men and women, this study demonstrated that individuals with the lowest COMT function (met/met homozygous) had the lowest capacity to activate μ-opioid receptor-mediated neurotransmission, albeit they had the highest number of μ-opioid receptors; the latter finding is consistent with upregulatory changes in the concentration of the receptors in the context of less efficient release of endogenous opioids. Heterozygous individuals demonstrated intermediate responses of the system and intermediate binding levels, and val/val homozygotes had the highest capacity to activate μ-opioid neurotransmission and the lowest level of receptors. These varying capacities to activate μ-opioid neurotransmission were further correlated with the capacity to suppress sensory, affective ratings of pain and the negative internal affective state induced by the stressor.[16]

What are these results teaching us about CFS and some of its associated symptoms, such as chronic pain, attention and concentration difficulties, difficulties in tolerating stress, and mood changes? Behavioral responses to a sustained stressor—in this case pain, but in the case of CFS maybe a persistent infectious process and changes in peripheral immunological factors—are regulated at the level of CNS neurochemical systems. These responses are interindividually variable, with both gender and genetic factors contributing to their short-term regulation. These variations may then contribute to the vulnerability or resiliency for development of pathological states, and the propensity to become further dysregulated over time. The regulation of stress, pain, and possibly other illness states involves brain regions also implicated in cognitive, emotional, and reward functions, which may then dysregulate as the injury or stressor progresses to a more persistent illness state. In this regard, alterations in opioid and dopamine receptor binding have been shown in chronic inflammatory and pain conditions.[19,20]

Correlations between COMT *val*^{158}*met* genotype and μ-opioid system activation in response to sustained pain—ventral pallidum and amygdala.



Association between COMT activity conferred by *met*^{158}*val* genotypes and μ-opioid system activation in response to the pain stressor. Shown are the mean ± S.D. values for the percent change in the magnitude of μ-opioid system activation in the ventral pallidum ipsilateral to the painful challenge and the contralateral amygdala (with some contribution of the adjacent anterior temporal cortex).

As a research agenda for CFS is developed, studies should be considered that address not only the immediate causes of CFS, but also the CNS mechanisms that become dysregulated during its persistence. Such mechanisms offer insight into the complex symptomatology of this illness and they also provide treatment targets for the symptoms.

Alterations in opioid and dopamine receptor binding have been shown in chronic inflammatory and pain conditions.

Discussion

Dr. Goldstein: Regarding the COMT polymorphism, biochemicals called catechol estrogens are substrates for COMT. It is possible that the link between pain perception and the COMT polymorphism may be independent of catecholamine metabolism, but also may be associated with the relative amount of available catechol estrogen. Most catecholamine metabolism takes place in the cells where it is produced. COMT is not expressed in most central neurons, and it does not play a major role in the inactivation of dopamine or norepinephrine in the brain.

Dr. Zubieta: Regarding the comment of COMT expression in central neurons, that is actually not the case. Effects of COMT polymorphisms have been shown on fMRI responses to cognitive stimuli, and variations in COMT activity have been linked to dopamine release in animal models, showing some sex differences. Animal models and humans also differ in terms of the contribution of COMT to dopamine metabolism. While in animal models COMT appears to be more involved in metabolizing dopamine and norepinephrine in cortical regions, in humans it appears to be strongly involved also in basal ganglia. There are some data in animal models on how the blockade or manipulation of COMT activity is associated with the release of monoamines in different areas of the brain. I am not certain whether that is related to catechol estrogen, but COMT is involved in metabolizing catecholamines in the CNS.

Dr. Goldstein: Our lab has the world's most sensitive assay for measuring metanephrines, the direct product of COMT. It has never been done, as far as I know, but if you think that COMT polymorphisms are altering catecholamine metabolism in a generalized way, then there is a direct testable hypothesis in terms of plasma levels of metanephrines.

Dr. Pocinki: I would like to suggest going back to an old methodology: physical examination. In seeing CFS patients over the years, and especially in the past year, I have been struck by the fact that all of the several hundred CFS patients have hypermobile joints. We know these individuals are predisposed to fibromyalgia and to tearing of periarticular muscles because of the stress on their joints. They are predisposed to exactly the type of muscle pain and joint pain without swelling or redness that is seen in CFS. Joint hypermobility is a sign of diffusely increased tissue elasticity. The patients' major problem is venous distension—their blood vessels are too stretchy, so they have pooling of blood and orthostatic intolerance. To compensate for their impaired circulation, these individuals produce more catecholamine.

These are the patients we saw in the Tahoe area who gave rise to the moniker "yuppie flu." They are the high-energy, always on the go, marathon-running, over-achieving people who run on adrenaline. When they try to go to sleep, they typically lie there for an hour waiting to fall asleep. When these people who have been running on adrenaline for years suddenly experience an infectious, environmental, or emotional insult, they lose their compensation. They stop making extra catecholamine. They describe exactly what our patients describe: someone has pulled the plug and drained out all their energy. They develop fatigue and problems with cognitive function. If they try to exercise, they rob their vital organs of even more circulation and they become exhausted.

Question: Is there any evidence that COMT inhibitors modulate pain perception?

Dr. Zubieta: We are beginning to study that now and will know more in about 6 months.

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The Cognitive Neuroscience of Fibromyalgia

Denise Park, Ph.D.

Cognitive aging is a plausible model for fibromyalgia because fibromyalgia patients have substantial memory and cognitive complaints. Normal adults have reliable memory function and have particular sensitivity in the hippocampal and frontal lobe systems. Some important neurochemical pathways, primarily the HPA axis, are disturbed in fibromyalgia patients and are similarly disturbed in older adults. A neurochemical mechanism exists that could mediate cognitive complaints. Believing they had identified a plausible mechanism to account for some of the cognitive complaints of fibromyalgia patients, we developed a study that hypothesized that fibromyalgia patients might have the cognitive function of adults 20 to 30 years older. Data collected on cognitive aging included three measures of speed of processing (how fast individuals process information) and four measures of working memory (how much information individuals can hold in their memory and manipulate in consciousness at any given time). Both are measures of long-term memory in which individuals are provided with lists of words or symbols and are asked to remember and recall them later. The measures are a proxy for word knowledge or vocabulary measures.

On reviewing our findings, we identified reliable declines across the life span in processing capacity abilities and in many modalities and domains. Notably, the data showed nothing remarkable about turning 60, 70, 50, or 30 years old. Beginning in the 20s, slow regular declines are seen across the life span. (Olympic athletes 30 and 40 years old are like 20-year-olds.) The brain is part of the body and, like every other body part, it ages gradually and almost imperceptibly. However, with age comes wisdom; individuals continue to accrue knowledge and expertise as they age. In addition, there is little evidence that as people age they become less effective, for example, in their jobs. Age-related changes may be observable only in occupations that involve heavy, constant processing demands, such as air traffic control.

How are fibromyalgia patients different from age-matched controls in their cognitive function? A quick review of the literature elicited few studies on the topic, and most were underpowered. All of the existing studies, even those that concluded that there was no difference, showed that the fibromyalgia patients consistently performed more poorly than the controls, although not significantly so. Subject matching was poor, and data were insufficient to suggest differences.

We recruited three groups of subjects and investigated their speed of processing, capacity, long-term memory abilities, and category fluency. Subjects were screened for depression and were excluded if they had anything more serious than mild depression. Drugs that could affect memory function were washed out for 2 weeks. The study included 23 subjects in each condition and 22 older controls; the mean age of the fibromyalgia group was 47 and the mean age of the older controls was 66. Education was matched precisely among the groups. There were no systematic differences in the samples. Subjects were always tested in triplicate, so that once a fibromyalgia patient was found, the research team searched until it found the perfect control.

Cognitive aging is a plausible model for fibromyalgia because fibromyalgia patients have substantial memory and cognitive complaints.

A score of 19 indicates mild depression on the Beck scale; a score of 15 indicates mild depression on the geriatric depression scale. The fibromyalgia patients in the study were slightly more depressed than the age-matched and older controls, but their scores fell below mild depression. They had mildly negative affect but were not depressed. The fibromyalgia patients self-reported more pain than the older controls or the age-matched controls.

To measure speed of processing, subjects were shown pairs of symbols and were asked to answer as rapidly as possible whether the two symbols were the same or different. The number of items an individual can complete in 2 minutes is an extraordinarily sensitive measure of how rapidly he or she processes information; it predicts variance on many cognitive tasks and is one of the most powerful measures of cognitive function. Fibromyalgia patients performed as well as the age-matched controls, but the elderly adults performed more poorly than the young adults, as expected. The mean age of the young adult subjects was 48, so the group did not include extraordinarily young people, such as college students. The fibromyalgia patients performed exactly like the younger adults.

Next, we measured working memory. For example, in one task, subjects were asked to listen to a sentence and to process, store, and recall information from the sentence. The researchers noted how many consecutive questions the subjects could answer without making an error. This difficult task represents an accurate measure of available processing capacity. On the measures of working memory, the fibromyalgia patients performed like older adults. Although the fibromyalgia patients performed as well as the age-matched controls in terms of speed of processing, they were significantly poorer and more like the older adults than the age-matched controls in terms of working memory capacity.

We then measured long-term memory capacity by giving the subjects lists of words to remember. The fibromyalgia patients performed like older adults and both the fibromyalgia patients and older adults performed more poorly than the age-matched controls.

As a measure of verbal fluency, the subjects retrieved from memory in 1 to 2 minutes as many words as they could that began with a particular letter. This task is age-sensitive; the ability to perform the task declines with age. The fibromyalgia patients performed similarly to the older adults, and both groups performed more poorly than the younger adults.

The fibromyalgia patients showed normal speed of processing, similar to age-matched controls; however, they performed significantly worse than age-matched controls and similarly to older adults on measures of working memory, verbal fluency, and long-term memory. The researchers also measured memory complaints, or meta memory, and found that fibromyalgia patients complained more about their memory than did other groups. The study suggests that fibromyalgia patients' memory complaints reflect a reality that their memory is age inappropriate, rather than a complaint bias. We also tried to correlate pain, depression, and anxiety with all of the memory measures; the only area that correlated was pain. Self-reports of pain correlated with memory function, but depression and anxiety did not.

The fibromyalgia patients showed normal speed of processing, similar to age-matched controls; however, they performed significantly worse than age-matched controls and similarly to older adults on measures of working memory, verbal fluency, and long-term memory.

Functional imaging is the ability to look in the brain at what components are being activated as a function of cognitive tasks. We developed a functional imaging technique to study fMRI and fibromyalgia in older patients. In an almost completed study, young adults and old adults were given a verbal working memory task and a PET scan. During the task, both young and old adults showed reliable activations in the left frontal cortex in the left hemisphere of the brain. At the top of the brain, good left hemisphere function occurred in both the young and old adults, but in the old adults, recruitment of the homologous areas and additional frontal regions also was seen. This finding emerges frequently in other studies: to perform a cognitive task, older adults recruit more of the brain in the frontal area, typically the homologous regions, than do young adults. Fibromyalgia patients showed cognitive function that is reminiscent of an older adult, but it was unknown whether they would show neural activations similar to those of an older adult.

To date, the data are based on 12 fibromyalgia patients and nine healthy controls, but we plan to include 15 fibromyalgia patients in our study. The subjects are matched carefully, and anything that could affect neural function, such as drugs, is washed out. The subjects are scanned on a 3T magnet at the University of Michigan using a spiral pulse sequence and then are given a working memory task: they see four letters on a visual display—such as S, B, G, and Q—and they are asked to quickly alphabetize the letters mentally while their brains are scanned. A 10-second slide allows their brains to come back to a resting state; it allows the hemodynamic response,

which is captured in fMRI, to return to baseline. We then observe what happens in the subjects' brains at different stages of the task and see the different parts of the brain that come into play. Although the time-course data are not yet in, data have been collected on the parts of the brain that were activated in the aggregate across the trial.

In the maintenance condition, subjects are shown four letters in alphabetical order and are asked to hold them in memory for 3 seconds, then they are shown a letter and asked if it is in the correct position. What is happening in these 3 seconds in the maintenance condition compared to the alphabetizing condition, in fibromyalgia versus control patients? We conducted trials with three letters and with four letters to see whether difficulty made a difference to fibromyalgia patients. There was little difference in activation between the three-letter and four-letter trials. Because brains are somewhat more active while alphabetizing than while maintaining, subtracting the maintenance image from the alphabetizing image leaves the residual activation of alphabetizing. The tasks for the study were designed not to be overly demanding, and the fibromyalgia patients and age-matched controls were given the same tasks to perform so that any differences in brain activation could not be attributed to differences in performance or to errors. This study resulted in equivalent performance between the fibromyalgia subjects and the control subjects; therefore, differences in neural activations between the groups cannot be attributed to differences in accuracy or to time on task.

It is likely that certain tasks are more demanding for fibromyalgia patients than for control subjects, so fibromyalgia patients engage more of the brain when undertaking those tasks.

Images of the fibromyalgia patients' brains in which the maintenance data were subtracted from the alphabetizing data included different views of the same activation from the right, top, and back of the head. There was bilateral activation in the midline medial frontal gyrus, with parietal activations in an area used for storage—the residual activation left while the subject is alphabetizing. Areas implicated in planning and storage showed enhanced activation in the fibromyalgia patients during the alphabetizing task. Similarly, a frontal area was activated in the left hemisphere—the analog to Broca's area, which is where language is processed in the left hemisphere. Activation in the right hemisphere was also seen during the alphabetizing task, in areas involved in rehearsal, in language, and in reasoning, providing evidence of bilaterality. We believe that we observed two-sided recruitment in both older adults and fibromyalgia patients during the alphabetizing condition, although additional images are needed to confirm this.

Control subjects appeared to have adequate capacity to perform the alphabetizing task, and little difference was observed in any parts of their brains when alphabetizing was subtracted from maintenance. Apparently, the alphabetizing task was less difficult for individuals with a lot of capacity, and therefore the fibromyalgia brains looked quite different from the control brains.

The researchers also subtracted the brains of the control subjects from the brains of the fibromyalgia patients, by stretching and moving the images slightly so they are in the same anatomical space and look anatomically identical. The fibromyalgia patients appear to show more planning and more need to prepare before responding. The significance of the medial frontal area, which is associated with eye movements and visual reflexes, is unknown in fibromyalgia. Activation was noted in the left thalamus, which is a gating area for pain and the site in which most information that reaches the cerebral cortex is processed. The right caudate is a central area responsible for preparation and selection of motor and emotional responses and formulation of strategies. The right inferior temporal gyrus and the medial temporal areas, which are involved in memory, were also evident on the scans. We will be recruiting more subjects and performing time-course analyses, which may reveal much more dramatic differences and which may provide a fuller picture of brain activity differences between the groups.

It is possible to subtract the fibromyalgia patients' data from the control subjects' data. In all slices of the brain, no significant activations in any slice or area of the brain were seen. The fibromyalgia patients showed more neural activation when using their working memory than did age-matched controls, with the primary areas being pre-motor areas (planning areas), thalamus (gating of pain), and caudate (strategizing and selecting emotional and motor responses). Some bilaterality was seen in the analyses in the frontal areas, reminiscent of aging. It appeared

that fibromyalgia patients need to activate more brain structures to alphabetize, and functional or structural systematic differences were encountered in the fibromyalgia patients.

Do fibromyalgia patients use different strategies because they are compensating, or have their brains been rewired so that functions have become structurally different? We believe the latter interpretation to be correct. It is likely that certain tasks are more demanding for fibromyalgia patients than for control subjects, so fibromyalgia patients engage more of the brain when undertaking those tasks.

It may be fruitful to compare fibromyalgia patients with older adults in a scanner and to collect data on brain volume as well as structure (e.g., size of different structures and amounts of neural tissue they contain). If fibromyalgia patients chronically dump large amounts of cortisol into their brains due to HPA dysfunction, it is plausible that such actions damage the hippocampus in a systematic way and result in decreased hippocampal volume, as in older adults. Such a decrease would not be associated with activation but rather with amounts of neural tissue (i.e., how many voxels comprise the hippocampus in a fibromyalgia patient versus an age-matched control).

This area of research is an interesting and important frontier for understanding cognitive dysfunction in fibromyalgia patients. Additional research is needed on long-term memory and vocabulary issues in fibromyalgia patients, as well as on the interface of emotion and cognition. Research is likely to reveal more dramatic responses to emotional stimuli in fibromyalgia patients, a promising research arena.

Discussion

Dr. White: I was particularly interested in the findings using fMRI that patients with fibromyalgia required increased activation of their brains for particular tasks. Were you able to measure the differential effect of the effort of performing different tasks, such as information processing versus memory? How much do you think the sense of effort affected the final results?

Dr. Park: The brain activation reflects that the fibromyalgia patients "work harder," using more of their brains. Brain activation is the purest possible measure of effort, particularly in the frontal areas. There are other areas of the brain that one would not think reflect effort. The parietal areas are storage areas, and the data suggest that fibromyalgia patients have to engage more parietal areas to store information, again suggestive of diminished capacity and increased effort.

Dr. Buchwald: You mentioned that you controlled for education, gender, depression, and other things. I did not notice whether you controlled for sleep. Both older people and fibromyalgia patients have poor sleep, and sleep is known to affect cognition.

Dr. Park: In the earlier study, we gave subjects Actigraph watches. We measured sleep quality in the fibromyalgia patients, the older adults, and the age-matched controls. We did not find any systematic differences; we did not find that sleep quality correlated with cognition.

Question: We obtained similar results in a similar study of CFS patients. We do not know whether neural activation drives cognition, although we assume it does. Some data suggest that blood flow is lower in patients with CFS and fibromyalgia. The next generation of neuroimaging studies could examine absolute blood flow, in order to connect brain activity with other systems that may be dysregulated in CFS patients.

Dr. Park: It is possible that different hemodynamic responses exist due to differentials in blood flow. We can assess that, because we have data on individual hemodynamic response functions, independent of the functional imaging data. We can examine the extent of normal function in this group of subjects.

Sex and Gender Issues in Multisystemic Illnesses

Margaret M. Heitkemper, Ph.D.

The methodological significance and complexities of sex and gender can be demonstrated using the model of irritable bowel syndrome (IBS). Gender differences exist in a number of common functional disorders, including fibromyalgia, migraine headaches, IBS, chronic pelvic pain, and temporomandibular pain. Chronic fatigue syndrome (CFS) and IBS are overlapping conditions. The two syndromes share a basic diagnostic approach in that they rely on symptom reports, in the absence of a clinically objective marker, to aid in diagnosis or to evaluate treatment effectiveness.

IBS is a common problem in the United States and worldwide; it is thought to exist in about 10 to 17 percent of the U.S. population. Among IBS patients, there are two to three women for every man. This sex-based differential exists in every country with the exception of India, which reflects either a sociocultural phenomenon related to healthcare access or exposure to different types of amoebae in the intestine. The diagnostic criteria for IBS are based on the Rome II criteria; the Rome III working group is re-examining these criteria, and new criteria may be forthcoming in the near future. Unfortunately, one survey found that only about 15 percent of primary care providers are aware of the existence of diagnostic criteria for IBS.

The etiology of IBS remains unclear, but a number of promising theories related to its pathophysiology are under investigation. The theories fall into three main categories. One is that people with IBS have visceral hypersensitivity (i.e., they are sensitive to normal stimuli in the bowel, such as gas or fecal material). A second theory is that IBS is related to alterations in gastrointestinal (GI) motility or transit. A more recent theory is that IBS is related to a prior infection or inflammation of the bowel; for example, people who have had enteritis resulting from food or water contamination are able to date their IBS symptoms to the enteral infection.

IBS can be used as a model of a multisystemic disorder because, in addition to lower GI symptoms, IBS features symptoms of the upper GI tract and bladder, gynecological problems, and somatic complaints. For example, about 70 percent of people with fibromyalgia also have IBS, and about 30 percent of people with IBS also have fibromyalgia. Recent studies have examined the overlap between IBS and gynecological conditions, particularly dysmenorrhea and premenstrual distress syndrome, which seem to occur more frequently in women who have IBS. Like the models for CFS, the model for IBS is complex, involving factors such as genetics and early childhood exposure to stress. The model includes factors that may trigger symptoms such as stress and diet and menstrual cycle changes, and response modifiers and outcomes involving the central, peripheral, and enteric ("little brain") nervous systems. Current IBS models address the brain-gut axis and the dysfunctional communication and interaction between the brain and gut.

Among IBS patients, there are two to three women for every man.

Issues of sex and gender in IBS fall into four major categories: stress response, menstrual cycle, affective symptoms, and sociological issues. Men and women are different in terms of stress reactivity and responsivity, and the menstrual cycle is an important confounding variable for many women. The overlay of affective symptoms also is significant, because people with IBS have higher rates of anxiety and depression. Sociological gender roles may affect symptom expression, perception, and healthcare-seeking behavior.

Because IBS is more prevalent in females, a disproportionate number of women are included in IBS studies; therefore, more is known about women with IBS than about men with IBS. Because the menstrual cycle may act as a symptom trigger, it is important to consider in which phase of the menstrual cycle women should be studied in IBS research; for example, whether comparison should be made at premenses or menses phases when symptoms are likely to be increased or at the follicular phase when symptoms are low. It is also important to consider oral contraceptive use, as well as hormone replacement therapy.

The higher prevalence of IBS in females appears at puberty; however, IBS is not well characterized in children. Recurrent abdominal pain, which occurs in children, appears to be similar to IBS. Children with this condition have chronic or recurrent abdominal pain that often keeps them from going to school, but diagnostic tests fail to reveal pathology. In children, the gender distribution of recurrent abdominal pain is equal, but as children approach puberty, it emerges predominantly in girls.

Since IBS is a symptom-based functional disorder, there is much debate about symptoms assessment. Some investigators believe that daily diaries are more reliable than retrospective recall of symptoms, such as pain, but retrospective recall may elicit the individual's worst experience. In our studies, the participants rate their symptoms on a daily basis and mark ovulation by use of a home luteinizing hormone surge kit, which allows for assessment of differences in the menstrual cycle phases. In comparisons of women's daily diaries with men's diaries, abdominal pain in women on non-menses days looked similar to abdominal pain in men; at menses, women had higher levels of pain. Women also reported more bloating, both before and during menses. Men report greater severity of diarrhea whereas women are more likely to report constipation.

Most studies on the physiological influence of the menstrual cycle have compared the mid-follicular (estrogen with no progesterone) and luteal (estrogen plus progesterone) phases. However, when symptoms are influenced by the menstrual cycle, they tend to be amplified in the late luteal and early menses phases, times of decreasing or low estrogen and progesterone. Additional work is needed using models of ovarian hormone withdrawal; however, the study of women at menses can be particularly challenging because of the co-occurrence of dysmenorrhea.

Regarding other symptoms, women with IBS report higher fatigue levels and greater sleep disruption than men with IBS. Using daily diaries, we measured how well women felt in the morning, how rested they felt, and how well they believed they had slept. Those items were then correlated with GI symptoms on the same day. The data showed a relationship between poor sleep and GI symptoms: when women had poor sleep the night before, they reported more symptoms the next day. The reverse was not true; GI symptoms during the day did not result in poor sleep that night. Although women with IBS subjectively report poor sleep, frequent awakenings, and feeling unrefreshed in the morning, there are few differences when objective sleep indicators are measured using standard polysomnographic measures of sleep. However, sleep stage differences do exist when comparing IBS women with and without mood disturbances.

Since IBS is a symptom-based functional disorder, there is much debate about symptoms assessment.

Few studies have examined the roles of estrogen and progesterone in GI function. Data from both humans and rodents indicate that estrogen slows gastric emptying rate. GI symptoms, such as heartburn and constipation, are common in pregnancy and appear to be related to progesterone or the combination of estrogen and progesterone.

Examining GI motility and transit during the estrous cycle in female rats in the laboratory is challenging. We removed the ovaries from female rats and implanted estrogen and progesterone pellets in order to examine the hormones' effects on gastric emptying and intestinal transit. Results confirmed that estrogen slows down intestinal transit and gastric emptying. The mechanisms accounting for these effects remain to be determined; the gut has receptors for estrogen, and estrogen may affect nitric oxide synthesis and release or act through ion channels. It is possible that GI symptoms in women are driven not by estrogen and progesterone but by the lack of testosterone; however, in a limited number of

studies, testosterone has not been shown to have a profound effect on bowel motility. Gonadotropin-releasing hormone agonists have been shown in small sample studies to be useful in treating conditions such as IBS, although there is concern about using such drugs over a prolonged period of time.

Currently, there is considerable support for the role of visceral hypersensitivity in the pathophysiology of IBS. Patients with IBS have increased sensitivity to balloon distention in the rectum. Both PET scanning and fMRI techniques have demonstrated that brain activation patterns differ between IBS and controls in response to balloon inflation. Gender differences in activation patterns have also been observed. Studies by Houghton and colleagues have shown that in non-symptomatic women without IBS, no differences in visceral perceptions are seen across the menstrual cycle; however, in women with IBS, differences in visceral hypersensitivity are seen across phases of the menstrual cycle. The time of high sensitivity or high pain perception is at menses. To test whether people with visceral hypersensitivity are hypersensitive to pain in general, several laboratories have used somatic pain challenges, including hand ice water immersion. Some have shown no differences between IBS and controls, while others have shown that IBS patients have increased somatic pain sensitivity.

Once serotonin has been turned on, other chemical mediators—for example, calcitonin gene-related peptide—are likely involved in affecting motility of the bowel.

Stress has also been identified as a trigger for IBS symptoms. Gender differences have been found in relation to the stress response, that is, men tend to respond with greater sympathetic nervous system activation. It has been proposed by Taylor that women have a "tend and befriend" pattern of response to stress. We began to look at the autonomic nervous system in IBS patients using several approaches. First was to look at catecholamine secretion in the urine. Women were asked to void before they went to bed and to provide first-voided urine specimens in the morning. In the morning, women with IBS had higher levels than controls of catecholamines and cortisol. Based on that result, we began to explore other indices of autonomic nervous system functioning including laboratory testing, for example, expiration/inspiration ratio, Valsalva, and postural changes. Overall, no differences were found in these reactivity measures between individuals with IBS and those without. Next, women in the study wore 24-hour Holter monitors to record the rhythms of their heart rates in a naturalistic environment. Differences emerged between IBS and non-IBS individuals, especially during the nighttime hours when the women had low levels of heart rate variability (vagal tone). Individuals typically have the highest levels of vagal tone during sleep, but those with IBS exhibited a dampened response. Among IBS subjects categorized by pain experiences (time of pain and severity of pain), women whose pain was rated as severe and unrelated to eating had the lowest level of vagal tone. Because it is known that psychological states can influence autonomic nervous system balance indicators, the subjects were also compared for history of depression and anxiety. Individuals who had a history of panic disorder, agoraphobia, depression, or dysthymia exhibited low levels of heart rate variability (lower vagal tone) compared to individuals with no history of mood disorders. When examining a multisystemic functional disorder, such as IBS, researchers should be aware of the coexistence of other conditions, such as depression, and their impact on physiological responses.

IBS, CFS, and related disorders are disruptive to individuals, and numerous studies have demonstrated reduced quality of life in those who have these conditions. Women with IBS report bloating, clothes not fitting, being unable to zip their pants, having the sensation of weight gain and fullness, and urgency. The psychosocial implications of these symptoms on body image perception remain largely unexplored. The disturbance in body image may explain the greater healthcare-seeking behavior noted in women than in men.

Future directions for research encompass several areas. Is there a common pathophysiology that underlies conditions such as CFS and IBS? Is that pathophysiology related to serotonin or another neurotransmitter, to an immune alteration, to autonomic nervous system dysregulation, or to hypothalamic-pituitary-adrenal (HPA) dysregulation? Does reproductive status or cycling make a difference?

While serotonin is important in the brain, most serotonin in the body is housed in the GI tract, including enteric neurons. Serotonin also is found in enterochromaffin cells, which are closely linked with the mucosal surface and nerve endings. In the gut, serotonin can play a role in pain transduction and transmission. There are a variety of serotonergic receptor subtypes and mediators in the bowel, as in the brain. Once serotonin has been turned on, other chemical mediators—for example, calcitonin gene-related peptide—are likely involved in affecting motility of the bowel.

Studies of serotonergic agents to manage IBS show that these drugs, at the doses studied, work in women but are less effective in men. The U.S. Food and Drug Administration has approved the serotonergic drugs for women, which underscores the possibility of basic differences between men and women, either in serotonin release, receptors, or metabolism. Related IBS research is investigating polymorphisms in the 5-HT3 receptor and the SERT promoter. Other models of IBS, including the post-infectious model, represent additional avenues for further research. This link involves further exploration of the role of inflammation in the initiation or persistence of IBS symptoms. Recent clinical advances have occurred in understanding IBS treatment with probiotics. Studying the systemic effects of changing the bowel flora, including activation of cytokines in the GI tract, may reveal effects reaching beyond the bowel.

A number of important areas should be explored, including the role of early environmental exposure in the evolution of disorders, such as IBS and CFS, and the possibility of genetic predisposition to those disorders. Autonomic nervous system and HPA alterations may be important predictors in individuals with severe symptoms.

Cognitive behavioral therapies are effective for treating disorders such as IBS and CFS; however, while these therapies can reduce symptoms, they do not change the basic underlying pathophysiology. We recently completed a study of cognitive behavioral therapy for women with IBS. The therapy reduced symptoms, reduced healthcare utilization, and increased quality of life, but it did not affect autonomic nervous system balance or catecholamine levels.

Discussion

Dr. Goldstein: Regarding catecholamine systems, adrenaline or epinephrine is a potent inhibitor of gut motility. The first bioassay preparation by Walter Cannon almost a century ago, about the effects of emotion on adrenaline release, depended on adrenalin relaxing the gut. In concept diagrams linking the adrenal system with the gastrointestinal tract, it is important to include that inhibitory effect of adrenaline. In addition, most of the dopamine made in the body is not made in the brain or the autonomic nervous system but in the gut. This dopamine appears to be part of a third type of catecholamine system. It is neither a neurotransmitter nor a hormone, but rather an autocrine/paracrine substance—something that is made in, released by, and acts on cells locally. In dealing with catecholamine systems, it might be worthwhile to explore the effect on IBS of drugs that act on dopamine receptors.

Dr. Heitkemper: I agree with your point. These agents have not been explored.

Dr. Van Konynenberg: In the past few years, Mark Pimentel at the University of California, Los Angeles, has published papers that support the hypothesis that IBS is caused by bacterial overgrowth of the small intestine, resulting from the lack of the cleansing peristaltic waves that a normal person has about 2 hours after eating. Does your work relate to why those waves might not be stimulated in people with IBS?

Dr. Heitkemper: We have not studied colonic motility in patients with IBS. The results related to normalization of motility following antibiotic treatment are interesting, but it is unknown whether these results can be sustained over time. The other question this brings up is whether gender differences or menstrual cycle differences may account for either the gender differences in migrating motor complexes or the amplification of symptoms in women around menses.

Dr. Baraniuk: Dean Befus at the University of Alberta has conducted some interesting studies on sympathetic innervation causing the release of gut hormones that have CNS and immune effects.

Dr. Heitkemper: The weakness of our methodology is that we do heart rate variability while looking at a gut-related condition. Ideally, we would monitor parasympathetic innervation of the upper and lower GI tract, but at this time that is not possible with our methodology. We have not explored the release of gut hormones in IBS. The vagus does innervate the upper part of the gut, but there are different branches, and the question is whether they react the same way. We have contemplated measuring pancreatic polypeptide, which is linked to autonomic nervous system function.

Most of the dopamine made in the body is not made in the brain or the autonomic nervous system but in the gut. This dopamine appears to be part of a third type of catecholamine system. It is neither a neurotransmitter nor a hormone, but rather an autocrine/paracrine substance—something that is made in, released by, and acts on cells locally.

Family Studies in Fibromyalgia

Lesley M. Arnold, M.D.

At the time we conducted a family study to test the hypothesis that fibromyalgia aggregates in families, three prior studies had reported preliminary evidence for aggregation of fibromyalgia in families, but the studies were limited by small sample sizes and lack of a comparison group. Our study used a case-control design in which the case families had a member (proband) with fibromyalgia and the control families had a member who did not have fibromyalgia but had rheumatoid arthritis. Patients with rheumatoid arthritis were chosen as the comparison group for several reasons:

- Rheumatoid arthritis is also a chronic pain condition that predominantly affects women.
- We wanted to draw subjects from the same study base, and both proband groups were recruited from the same rheumatology outpatient clinic.
- We wanted to reduce the possibility of ascertainment bias due to the treatment-seeking effect for a chronic pain condition.

A second hypothesis was that fibromyalgia in a proband is associated with increased tender points and higher levels of pain in relatives. Fibromyalgia is defined not only as chronic widespread pain, but also as the presence of tender points. We decided to investigate whether tender points themselves also aggregate in families, measuring tender points using a dolorimeter, which provides a measure of pressure-pain thresholds.

The third hypothesis was that fibromyalgia co-aggregates with major mood disorder (major depressive disorder or bipolar disorder) in families. Hudson and colleagues discovered that fibromyalgia was associated with a high familial rate of mood disorders. However, the high prevalence of mood disorders in families of patients with fibromyalgia could have been due to the familial aggregation of mood disorders. We addressed the methodological limitations of the prior family studies and controlled for the effects of major mood disorders in probands to confirm the co-aggregation of fibromyalgia with major mood disorder in families.

We were also interested in gathering preliminary data on the co-aggregation of fibromyalgia with other disorders that are thought to be related to fibromyalgia, including chronic fatigue syndrome, irritable bowel syndrome (IBS), migraine, and anxiety disorders.

In the design of the family study, the age range of the probands was restricted to 40 to 55 years of age so that first-degree relatives would be approximately the same age within the type of relative (child, sibling, and parent), and between proband groups, and because both age and cohort effects exist in the prevalence of major mood disorder and possibly also in fibromyalgia. At least one relative was required to be available for evaluation.

Diagnostic procedures included the Structural Clinical Interview for DSM-IV (SCID) for assessing lifetime psychiatric illness, with supplemental modules for fibromyalgia, CFS, IBS, and migraine. A revised version of the Family Interview for Genetics Study (FIGS) was used with relatives to elicit information about the non-interviewed first-degree relatives. The tender point exam used a Fischer dolorimeter. The myalgic score was the sum of the dolorimetry pressure-pain threshold results for all 18 tender point sites, and the tender point count was determined by the number of tender points with a threshold of <4 kg/cm^2. Pain was assessed with visual analog scales that determined pain right now, worst pain during the past 6 months, and average intensity of pain during the last 6 months.

To evaluate the familial aggregation of fibromyalgia, we used a univariate predictive logistic regression model, with the presence or absence of fibromyalgia in a relative as the outcome, and the presence or absence of fibromyalgia in the proband associated with that relative as the predictor. To test the co-aggregation of major mood disorder with fibromyalgia, we used a multivariate logistic regression model, with bivariate disorder status of a relative (the presence or absence of fibromyalgia and major mood disorder) as the outcome and bivariate disorder status of the corresponding proband as the predictor. The model estimates an aggregation odds ratios for the familial co-aggregation of fibromyalgia with major mood disorder, while controlling for the effects of the familial aggregation of fibromyalgia, the familial aggregation of major mood disorder, and the co-occurrence within persons of fibromyalgia with major mood disorder.

We gathered information on more than 500 relatives of the probands with fibromyalgia and on more than 200 relatives of the probands without fibromyalgia, and interviewed and examined a subset of those individuals.

Table 1. **Lifetime Prevalence of Selected Disorders in Relatives of 78 Probands with Fibromyalgia and 40 Probands without Fibromyalgia**

	Relatives (n=533) of Probands with Fibromyalgia, N (%)	Relatives (n=272) of Probands without Fibromyalgia, N (%)
Fibromyalgia	34 (6.4)	3 (1.1)
RA	4 (0.8)	6 (2.2)
IBS	44 (8.3)	11 (4.0)
Migraine	43 (8.2)	12 (4.4)
Migraine with aura	27 (5.1)	9 (3.3)
CFS	5 (0.9)	1 (0.4)
Major depressive disorder	157 (29.5)	50 (18.3)
Bipolar I	7 (1.3)	1 (0.4)
Bipolar II	7 (1.3)	1 (0.4)
Any major mood disorder	171 (32.1)	52 (19.1)

The presence of lifetime comorbid diagnoses for the fibromyalgia and rheumatoid arthritis probands was typical for these patients in tertiary care settings. Major mood disorder, anxiety disorders, CFS, IBS, and migraine were more common in the probands with fibromyalgia than in those with rheumatoid arthritis.

Fibromyalgia was found to aggregate strongly in families. The estimated odds of fibromyalgia in a relative of a proband with fibromyalgia were 8.5 times the odds of fibromyalgia in a relative of a proband with rheumatoid arthritis (P = 0.0002). Only three cases of fibromyalgia were seen in the male relatives overall; therefore, aggregation was driven primarily by the effect of the female relatives.

The relatives of probands with fibromyalgia displayed significantly higher levels of tenderness—both increased tender points and decreased myalgic score—than was displayed in the relatives of probands with rheumatoid arthritis. There was also a trend toward higher levels of pain in relatives of fibromyalgia probands, and male relatives were significantly less tender than were female relatives. However, there was no significant group-by-sex interaction; males in the fibromyalgia proband group were more tender than males in the control proband group and the same was true for females.

Table 2. **Estimated Difference Between Groups* on Pain Measures**

	Estimate	95% CI	*P*
Tender point score	34.4	19.5,49.3	<0.0001
Myalgic score	-17.9	-26.8, -8.9	<0.0001
Pain, current	16.0	-2.7,34.8	0.094
Pain, worst	12.4	-4.3,29.1	0.15
Pain, 6 months	18.0	-1.6,37.6	0.072

*Relatives of probands with fibromyalgia vs. relatives of probands without fibromyalgia; difference in ranks for all measures except myalgic score, which is difference in means; adjusted for age, sex, type of relative, and correlation of observations within families.

Fibromyalgia co-aggregated significantly with major mood disorder (odds ratio = 1.8; P = 0.013). No significant co-aggregation was found for the other disorders of interest, possibly because the study was underpowered for that purpose, but some trends were observed: anxiety disorders and eating disorders were more common in the relatives of probands with fibromyalgia than in relatives of rheumatoid arthritis probands, but there was no difference in the prevalence of substance use disorders between the relative groups.

The finding that fibromyalgia aggregates in families has important clinical and theoretical implications. Familial aggregation of fibromyalgia supports the validity of fibromyalgia, which is currently diagnosed with criteria that were developed by expert consensus and limited by the lack of definitive laboratory or

pathological findings. In addition, familial aggregation of fibromyalgia suggests a possible genetic contribution to the etiology of fibromyalgia. The study also revealed that fibromyalgia in probands was associated with increased tender points and decreased myalgic scores in relatives and that this association was independent of the presence of fibromyalgia or major mood disorder in the families. These findings support the validity of pressure-pain thresholds in fibromyalgia and suggest that there may be inherited factors in pain sensitivity. The co-aggregation findings suggest that fibromyalgia shares some familial factor or set of factors with mood disorders, but also has a factor or factors that are independent of mood disorders. The data are not consistent with the hypothesis that fibromyalgia is simply caused by mood disorder; rather, they are consistent with the hypothesis that fibromyalgia and mood disorders share important common and possibly heritable causal factors.

The study's limitations included low power to detect co-aggregation with other disorders of interest; additional research with more subjects is needed. Familial aggregation or co-aggregation does not prove underlying genetic factors, although it is suggestive. Our next steps are to continue to recruit families to allow for greater power to test the co-aggregation of other disorders and to re-examine the issue of pain in relatives by using more sensitive measures or by recruiting more families. Fibromyalgia may be caused in part by genetic factors, which may be shared with other disorders, particularly mood disorders.

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The finding that fibromyalgia aggregates in families has important clinical and theoretical implications.

Dr. Arnold also presented results of a genetic study of fibromyalgia under review for publication that could not be included in this report.

Biomarker Discovery in Illnesses with No Lesions

Suzanne D. Vernon, Ph.D.

An ideal study design system is needed that standardizes the assessment of a particular illness. Using instruments in a similar fashion should enable comparison of studies across study sites. If samples are collected, processed, and examined in the same way, it should be possible to interpret the data and reach a greater understanding of the illness.

Biomarker discovery involves finding something in a sample or an individual that defines or identifies a disease. Good biomarkers and knowledge of where and in what part of the lifespan the biomarker is found have the potential to increase understanding about disease pathogenesis. There are a number of places in a disease process to discover biomarkers that would help provide an understanding about susceptibility, the dose of the biomarker, the mode of action of that biomarker, and the disease pathogenesis and etiology.

It is unlikely that illnesses such as chronic fatigue syndrome, irritable bowel syndrome (IBS), and many neuroendocrine disorders will have a single biomarker, molecule, or gene to explain the whole illness. Many factors will likely be involved in any illness process including genes, gene expression, environment, and behavior.

CFS is a complex illness. The Centers for Disease Control and Prevention's (CDC) approach to studying CFS is highlighted and briefly reviewed in the Chronic Fatigue and Immune Dysfunction Syndrome Association of America's newsletter in an article entitled "CDC Shifts to High-Tech Clinical Studies." CDC's CFS program is an integrated multidisciplinary program that seeks to identify the number of people who suffer from CFS and then to enroll these people in studies that seek to define the illness. We now know that the number of people with CFS in the United States ranges from 500,000 to 1 million adults.

In order to accurately determine the incidence of CFS, it is necessary to define the illness. A case definition was developed in 1988 and further refined in 1994; a process is underway to refine the definition even further by using standardized instruments and similar study designs so that any case definition can be based on empirical information rather than on consensus. Once people with CFS are identified, samples are collected from them in an attempt to decipher the pathophysiology of the disease, seeking to identify causal agents or risk factors as well as biomarkers or diagnostic markers. It is important to identify a good biomarker for CFS so that the illness is identifiable and is recognized as a legitimate illness by healthcare providers.

It is unlikely that illnesses such as CFS, IBS, and many neuroendocrine disorders will have a single biomarker, molecule, or gene to explain the whole illness.

The CDC also attempts to educate people about CFS. When more people believe that CFS exists, a more concerted scientific, research, and medical effort to understand and treat the disorder will become possible.

The CDC's CFS research program uses population-based studies, model studies, and clinical studies. These studies include a population-based study that has been underway in Wichita during the past 5 years, an exercise model study, a post-infected fatigue model study, interferon alpha challenge studies, treatment studies, hepatitis C studies, and a study of interleukin-6. Each of these studies integrates microbiology, immunology, endocrinology, neurology, epidemiology, genomics, and proteomics to develop an understanding of the illness.

The CDC is currently conducting gene expression profiling studies for biomarker discovery and to further our understanding of disease pathogenesis. The Wichita population-based study is a random-digit-dialing telephone survey of about 40,000 households. According to the U.S. Census Bureau, Wichita's demographics represent the U.S. population in general: the distribution of various races, ethnicities, and sex is represented in Wichita. This study began in 1997 and was designed to estimate the prevalence of CFS and other fatiguing illnesses in the population. Individuals identified by phone as having fatigue were followed up with a more detailed interview. If it was determined that the individuals had a chronic fatigue-like illness, they were invited to come in for clinical participation and were followed over time. Over the course of the study, 90 people with CFS were identified. A random sample of non-fatigued individuals was also brought into the clinic—incident cases or cases that were misdiagnosed originally at baseline. The study results are generalizable to the U.S. population.

A post-infected fatigue study involves Lloyd's cohorts from the University of New South Wales in Australia. It is a longitudinal study of acute infection with Epstein-Barr virus, Ross River virus, or Coxiella (a rickettsial agent that causes Q fever). All three infections are known to result in a chronic fatiguing illness. Ross River virus, like influenza, is an RNA virus. Specific to Australia, Ross River virus is a typical arbovirus that is carried by mosquitos, resulting in a fatiguing illness with arthralgia and myalgia. These three agents infect different types of people: the Epstein-Barr cohort is composed predominantly of young men and women, the Ross River virus affects a mix of people throughout the Australian continent, and Q fever predominantly affects men working in the animal processing industry. The subjects were enrolled based on acute infection and an immunoglobulin M response, and then are followed over time. Currently, many of the subjects have been followed for up to 3 years post-infection.

If a gene is actively being expressed in the PBMC sample, it will hybridize to the corresponding gene spot on the microarray.

Another CDC study conducted with Jones at National Jewish Hospital is an exercise challenge study that involves challenging patients with a stationary bicycle and examining blood samples before and after the challenge.

One of the biggest hurdles to studying CFS is that there is no known lesion; nothing anatomically accessible can be biopsied. We had to make a decision on what type of sample would be representative of CFS or would be potentially useful for biomarker discovery. We chose to study peripheral blood as a representative sample of CFS. The rationale for that choice was that, because blood circulates through the body continually, cells and serum could serve as sentinels of various disease processes. Cells carry signals throughout the body and various organs, and serum bathes all the organs of the body and contains anything that the organs do not want, which could be an indicator of various disease processes.

All of our genomics studies use the peripheral blood mononuclear cells (PBMCs). The genomics approach we use to profile gene expression is called microarray technology. Microarrays are glass microscope slides spotted with thousands of cDNAs or oligonucleotides. A probe made from the subject's PBMC sample is put on the microarray and allowed to hybridize. If a gene is actively being expressed in the PBMC sample, it will hybridize to the corresponding gene spot on the microarray.

This hybridization reaction is detected with a sensitive gold and white light system; spots that appear bright are clearly positive and those that cannot be seen are obviously negative. This technique is a rich and relatively low-tech approach that derives much information from one sample.

The reason we are relatively comfortable and confident with using PBMC samples for gene expression profiling and biomarker discovery is that a number of studies have shown that PBMCs in both normal and disease states traffic back and forth through the blood-brain barrier, either through primary signaling or secondary messenger systems. The great dynamic between what happens in the body and what happens in the head may allow researchers to decode disease processes.

By using gene expression profiling of the peripheral blood, we have been able to differentiate individuals with and without CFS. Recently, we have also been able to demonstrate with gene expression profiling that CFS is a heterogeneous illness. Dr. Toni Whistler in our group examined samples from 25 CFS subjects in the Wichita study—all were women in their 40s—and attempted to use gene expression profiling to determine whether the CFS subjects could be distinguished. Our hypothesis was that since symptoms define CFS, these symptoms should correlate with specific gene expression patterns; the result was that they did not. One group of subjects had sudden onset and another group had gradual onset of illness, and researchers identified only 112 out of 4,000 genes that were different between the two groups. Fifty percent of the genes that were upregulated in the rapid onset individuals were metabolism genes, although a number of different functional families were seen, including immune system genes and cell adhesion.

Can the brain be profiled by looking at the blood? An approach taken by the CDC to answer that question involves an informatic query of blood cDNA databases. Researchers identified 1,800 genes involved in psychoneuroendocrine immune pathways, in an attempt to determine what occurs with the HPA axis and the brain by looking at the blood. They created a normal blood cDNA database that contained 45,000 genes and queried it for 1,800 genes, finding that about 1,400 genes are expressed in the blood. Of these genes, 24 percent are endocrine, 14 percent are neurotransmitters, 40 percent are immune, and 22 percent are involved in various aspects of other functions. About 5 percent of the genes are neurotransmitter genes expressed in their PBMCs. The CDC is optimistic that peripheral blood will provide some indication of what occurs in the brain. Peripheral blood expression of hormone receptors was found—the hormone responsive protein ZNF-147 and several GABA-nergic system proteins.

To attempt to predict who will not recover from acute infection with Epstein-Barr virus, and because of a metabolic abnormality in some CFS subjects, the CDC gene expression databases were queried for metabolic genes. Three years of data are available on those individuals, so it was possible to discern who recovered and who stayed sick. The subset of genes that separates the sick individuals from the recovered individuals falls into metabolic pathways—mitochondrial transport and lipid metabolism genes.

The CDC's work can be credited to a comprehensive multidisciplinary approach that includes researchers within and outside the CDC, including collaborators from Emory University, National Jewish Hospital, Australia, Karolinska Institutes, University of Miami, Chronic Fatigue and Immune Dysfunction Syndrome Association, APS Associates, University of Texas MD Anderson Cancer Center, and an international group that has been working with the CDC on the CFS case definition.

Discussion

Dr. Natelson: As a believer in the brain being one of the organs involved in CFS, I have recently obtained preliminary findings from spinal fluid. The brain probably is involved in CFS, which should be considered when choosing a body fluid to study. It also is important to focus on fMRI.

Dr. Vernon: I agree, but it is difficult to obtain spinal fluid. While it can be done in small numbers at relatively few sites, it is difficult to do on a population scale. Blood, however, can be obtained and processed relatively easily. It will be incumbent upon us to be able to decode the information we find and relate it to what occurs in the brain. We hope that through animal studies we will be able to look at gene expression in the brain and blood of rats and see if they correlate and, if not, determine how to decode that information.

Dr. Buchwald: You studied most of the people who reported an acute onset a long time after that onset, and apparently the metabolic genes were turned on. The individuals may have been sick for 6, 8, or 10 years, and it is questionable whether the acute onset is reliable.

Dr. Vernon: My rationale was to ask whether or not we can predict who is most susceptible to developing a chronic fatiguing illness when they get Epstein-Barr virus or some kind of chronic or acute stressor. It was proposed as a quick, hypothesis-generating query. I looked at a number of our favorite hypotheses. For example, in people I knew who were acutely ill and did not get better, I could not find any differences in clustering when I looked at some of the cytokine receptors, despite some evidence of downregulation of the receptors. When I queried some of the metabolic pathways, I started to see differences. Perhaps subtle differences predispose us to certain types of stressors or infections and make it difficult to recover.

Session Five: Therapeutics

Chair: Dimitris A. Papanicolaou, M.D.

Regardless of its clinical significance and regardless of the symptoms and disability it causes, chronic fatigue syndrome (CFS) has no cure at this point. CFS is multifactorial and likely represents more than one disorder. The literature reveals studies that have used pharmacological treatments and a few studies that have used nonpharmacological treatments; all were met with variable success. It is not yet possible to state that one treatment works and another one fails, but it is possible that some treatments will work with some groups of people.

Innovative Treatments in CFS

Daniel Clauw, M.D.

Individuals who went to the first Gulf War and returned with otherwise unexplained pain, fatigue, and other symptoms have something similar or identical to chronic fatigue syndrome (CFS), fibromyalgia, or multiple chemical sensitivity somatoform disorders. Studies that have been conducted on Gulf War illnesses, fibromyalgia, and CFS during the last 3 years have provided insight into what does and does not work in treating these diseases.

The Veterans Administration Cooperative Trials Network is a significant resource for conducting clinical trials. Researchers design a trial and then the Cooperative Trials Network conducts the trial; researchers only need to design the trial and analyze the data at the end.

Nonpharmacologic Therapy

Cognitive behavioral therapy and exercise have been shown to work well in single-site efficacy trials. In moving from an efficacy design to an effectiveness design, a major issue is whether these therapies work well in routine clinical practice for individual patients.

Many drugs have been shown to be effective in short-term, single-site trials conducted at academic medical centers. However, studies conducted at academic medical centers in many cases do not follow good clinical practice and do not have in place the kind of stringent monitoring requirements and other compliance issues that characterize trials that hope to garner FDA approval. Phase II and Phase III trials conducted in the context of an investigational new drug are generally conducted in a more stringent manner than other types of studies.

A large study conducted within the Veterans Administration (VA) and the Department of Defense (DoD) healthcare system, published recently in *JAMA*, involved about 1,100 veterans of the first Gulf War who were being seen in 18 VA and two DoD health care centers. About 700,000 U.S. troops were deployed to the first Gulf War, with few combat-related casualties. Within 6 to 12 months, people who served in the war began to return and to complain of difficulty with memory, pain, fatigue, and several other symptoms. The symptoms that Gulf War veterans suffer represent the same clusters of symptoms that occur in the general population and are called fibromyalgia and CFS. The term coined by the Centers for Disease Control and Prevention (CDC) to describe this constellation of symptoms and syndromes—as it occurred in Gulf War veterans and as it occurs in the general population—is chronic multisymptom illnesses (CMI).

Although CMI came to the forefront after the first Gulf War, military historians in the United States and the United Kingdom have noted that after every war since the mid-1800s—as far back as reliable records exist—there have been soldiers who returned with otherwise unexplained pain, fatigue, and memory problems. After every war, this constellation of health problems has been called something different: Da Costa's Syndrome, soldier's heart, effort syndrome, and shell shock. The primary symptoms are the same, no matter what these problems are called.

The symptoms that Gulf War veterans suffer represent the same clusters of symptoms that occur in the general population and are called fibromyalgia and CFS.

The U.S. Government has spent about $240 million looking at the effect of specific exposures in the theater of operations in the first Gulf War. One study implied that individuals who received vaccines at the time of deployment were more likely to develop symptoms than if that same set of vaccines had been

given 6 months prior to deployment. With the exception of that single study, no other study has linked any specific exposure in the first Gulf War to any of the symptoms of these post-deployment syndromes.

In 1999, the CDC defined CMI as comprising several factors: multifocal pain; fatigue severe enough to limit activities; and difficulties with cognition, memory, or mood. Sometimes cognitive and memory problems are separate from mood and sometimes they are linked together. Under this definition, 10 to 15 percent of the population of the United States meet the criteria for CMI. The aggregate prevalence of fibromyalgia (which affects approximately 4 percent of the population), CFS (affecting less than 1 percent of the population), and somatoform disorders (affecting 4 to 5 percent of the population) add up to about 10 percent of the population.

We hypothesized, based on previous data in fibromyalgia, CFS, and other chronic medical illnesses, that exercise and cognitive behavioral therapy (CBT) would lead to improvements in physical function in individuals with CMI. Furthermore, we hypothesized that adding CBT and exercise together would produce either additive or synergistic effects—people would show more improvement when the two therapies were combined than if they received either therapy separately.

Under this definition, 10 to 15 percent of the population of the United States meet the criteria for CMI.

The definition of CMI that we used to create inclusion criteria was that research participants had to be Gulf War veterans with two or more of the following symptoms: fatigue limiting usual activity, pain in greater than two body regions, and neurocognitive symptoms. These symptoms had to have begun after August 1990 (when the first Gulf War began), had to have lasted for more than 6 months, and had to be present at the time of screening. About 1,100 veterans satisfied these eligibility criteria and were randomized to one of four treatment arms: usual care alone, usual care plus CBT, usual care plus exercise, or usual care plus CBT and exercise.

Both CBT and exercise were administered in groups of three to eight. The sessions for both CBT and exercise were 60 minutes long, with the combined CBT and exercise group lasting 90 minutes. These treatments were administered for 3 months, but the primary outcome measure was the proportion of individuals who had a clinically meaningful improvement in their physical component summary score of the SF-36 at 1 year. Instead of the outcomes being measured at 3 months at the end of the trial, the primary outcome measure was examined at 1 year, looking for a sustained improvement in function after people had received these therapies.

Secondary outcome measures included pain, fatigue, cognitive symptoms, distress, and mental health functioning based on the SF-36. Because these were veterans, 85 percent of the subjects were men; their mean age was 40 years. Although they only needed two of three symptoms to meet criteria for CMI, 81 percent of the individuals had all three symptoms: pain, fatigue, and memory problems. The mean duration of symptoms was about 7 years, which was expected because the trial was started about 7 years after the end of the war.

Using Prime MD, a screening instrument for psychiatric comorbidities, we found that about 45 percent of the subjects had present major depressive episodes or dysthymia, 35 percent had an anxiety disorder, and 43 percent had post-traumatic stress disorder. In total, 65 percent of the individuals in this trial had one of these three disorders, and 65 percent of subjects either had pending disability status or were receiving disability payments.

Primary data analysis showed a modest improvement in physical function in the group that received CBT, but not in the group that got exercise alone. In the exercise-alone group, no difference was observed compared to usual care. In the two groups that received CBT, about 7 percent more of those individuals showed clinically meaningful improvement in physical function at 1 year. Exercise produced much more improvement in individual symptoms, whereas CBT led to less improvement in individual symptoms such as pain, mental health, and cognitive function. Exercise led to improvement in symptoms and CBT led to improvements in function but, surprisingly and disappointingly, no additive or synergistic effects between CBT and exercise were observed. Subjects who received both treatments were not any more likely to improve than were subjects who received one or the other treatment.

The effect sizes observed in this trial were significantly less than the effect sizes seen in previous

single-site efficacy trials of either exercise or CBT, in either fibromyalgia or CFS. Several interpretations are possible. The hypothesis could be wrong: perhaps Gulf War illness is not the same as fibromyalgia or CFS; however, many data suggest that it is at least very similar and probably identical to fibromyalgia and CFS. This patient population was unusual in that it contained a high proportion of people who were either presently on disability or had a pending disability status. When predictors of outcomes in this study were examined, it was clear that people who were either on disability or had pending disability claims were much less likely to respond to therapy. Given that 65 percent of subjects in this trial were in that situation, this variable had a powerful effect on study results, and the effect of being disabled likely overwhelmed any treatment effect in the study.

A number of compounds have been studied in fibromyalgia and CFS that, in one way or another, affect monoamines. Some drugs raise serotonin, some raise norepinephrine, some raise dopamine, and some raise a combination.

Another predictor of outcome was tender points, defined as a sedimentation rate for distress; in population-based studies, they are highly correlated with individual distress levels. Subjects who had greater numbers of tender points were less likely to experience improvement in their physical function, pain, or fatigue. It is likely that the relationship between tender points and unresponsiveness to therapy is a surrogate for distress being unresponsive to therapy.

Personality disorder was a statistically significant predictor of poor outcome with respect to physical function, although not in other domains. Presence of a personality disorder was a much stronger predictor of poor outcome than was the presence of a mood disorder. Presence of a psychiatric disorder or the number of psychological disorders was not predictive of unresponsiveness to therapy.

Conclusions of this study are: 1) CBT had a marginally significant impact on self-reported physical function in veterans with Gulf War illness; 2) exercise resulted in improvement in symptoms and; 3) there were no synergistic or additive effects. The magnitude of these changes was modest. This study also points out the differences between efficacy studies and effectiveness studies.

Pharmacologic Therapies

Use of staphylococcus toxoid in individuals who meet criteria for both CFS and fibromyalgia is innovative and unique. No one knows why this treatment may be working. An abstract and manuscript published in the *European Journal of Pain* in 2002 discusses this treatment. Individuals who met criteria for fibromyalgia and CFS were randomized to receive weekly injections for 6 months of either staphylococcus toxoid or placebo. The primary outcome was the proportion of individuals who noted a 50 percent improvement on a subscale of the Comprehensive Psychopathological Rating Scale. (This subscale was not commonly used, which is somewhat problematic.) In an intent-to-treat analysis, the researchers showed a 65 percent response rate in the active treatment group versus an 18 percent response rate in the placebo group. Since that time, the maker of staphylococcus toxoid has decided that it cannot guarantee uniform purity, so investigators are not likely to go further even though initial results appeared somewhat promising.

Pregabalin is a compound under development by Pfizer. Its method of action is not clear, but Pregabalin is structurally similar to Neurontin, a GABA (gamma-aminobutyric acid) penton. One study compared Pregabalin at 450 milligrams/day, 300 milligrams/day, and 150 milligrams/day to placebo. A large number of subjects were randomized to each arm of the trial; 1 week of titration was followed by 8 weeks of therapy. About 20 to 25 percent of the subjects withdrew. About 29 percent of the subjects (a statistically significant number) experienced a 50 percent improvement in pain in the 450-milligram dose, as compared to 13 percent of those in the placebo dose. The 450-milligram dose also led to statistically significant improvements in patient global improvement—whether subjects report they are better, unchanged, or worse than when they began the trial.

A number of compounds have been studied in fibromyalgia and CFS that, in one way or another, affect monoamines. Some drugs raise serotonin, some raise norepinephrine, some raise dopamine, and some raise a combination. Selective serotonin reuptake inhibitors raise serotonin. Another class of compounds that raise both serotonin and norepinephrine is the tricyclic compounds, some of which have almost equal effects on serotonin and norepinephrine, and some tricyclics are almost entirely

noradrenergic compounds. (Pharmacologically they are quite different, although they are often grouped together.) Monoamine oxidase (MAO) inhibitors and amphetamines generally act to raise both norepinephrine and dopamine.

A clinical trial reported in *Psychosomatics* in 2003 by Olsen and colleagues randomized 20 patients with CFS to receive either dexamphetamine or placebo and noticed statistically significant improvements in fatigue. No improvements in physical function or pain were seen. The sample size was so low that this study was not powered to detect more subtle improvements in the other domains. It was only intended to be and was published as a pilot study to suggest that a larger randomized control trial should be conducted to look at dexamphetamine and its effects on fatigue and physical function in people with CFS.

A study published a couple of years ago by Hickie's group in the *Journal of Psychiatry* randomized 90 individuals to receive moclobemide, which is one of the new classes of MAO inhibitors that is not yet available in the United States. Traditionally, MAO inhibitors have had many side effects, especially with respect to not being able to consume foods that include tyramine and leading to toxicity; this new class of MAO inhibitors is not as problematic. This trial showed that, in individuals with CFS, 51 percent of the subjects receiving the drug improved versus 33 percent of those receiving placebo. The researchers noted that the improvements were in energy and vitality; improvements in mood and pain were not seen. The MAO inhibitor at this dose had neither antidepressive nor analgesic effects.

Milnacipran is approved in about 25 countries as an antidepressant. It is a mixed serotonergic noradrenergic reuptake inhibitor and it weakly antagonizes N-methyl-D-aspartate receptors, although it is not clear that that is related to its mechanism of action. Milnacipran has a profile close to amitriptyline with respect to being a mixed reuptake inhibitor. The company conducting the Milnacipran study also decided to look at novel outcome measures in fibromyalgia. They posited that one of the reasons that many previous studies in fibromyalgia had not succeeded was because the outcomes had not been collected in the best way to show separation between drug and placebo.

In research on "ecological momentary assessments," instead of recording their symptoms in diaries, subjects wear a Palm-based or wristwatch-based device that prompts them randomly over the course of the day to record their pain, fatigue, and other symptoms. This technology produces extremely rich data sets compared to the data obtained with paper-and-pencil diaries, but it is expensive to collect data in this way. One of the reasons to use this new technology was discussed in a study published in the *British Medical Journal* by Stone and colleagues showing that compliance with paper-and-pencil diaries is abysmal. The subjects were asked to record the time they recorded their pain and pain level, and a microchip was installed in the cover of each diary, unbeknownst to the subjects. Even when Stone and his group gave subjects a 90-minute window for margin of error, only 11 percent of the diary entries could have occurred when the person said they occurred.

Subjects wear a Palm-based or wristwatch-based device that prompts them randomly over the course of the day to record their pain, fatigue, and other symptoms.

It has been known for a long time that people backward-fill diaries, but people also forward-fill diaries—they anticipate what their symptoms will be before their symptoms even occur. This is a real problem; the FDA is concerned about data integrity and has been looking at these types of technologies when they recommend going forward in different trials. This device is somewhat annoying to wear, but produces very good compliance with the diaries. Many researchers are considering moving toward these kinds of assessments, rather than using paper-and-pencil assessments.

The Milnacipran trial randomized people to placebo, once-a-day Milnacipran, or twice-a-day Milnacipran. Similar to the Pregabalin study, the investigators showed that the proportion of the subjects who had a 50 percent improvement in pain was about 37 percent in those subjects who received twice-a-day Milnacipran and 13 percent in the placebo group, a statistically significant result. Large differences in patient global improvement were also seen. The results were much better with either dose of Milnacipran than with the placebo.

One lesson learned in these studies is to enroll subjects with fibromyalgia or CFS who are in primary care. Tertiary care patients have high levels of distress, high levels of mood disorders, high numbers of maladaptive illness behaviors, and multiple disability and compensation issues; they also are recalcitrant to therapy. At the other end of the spectrum, recruiting out of the general population would include many people who are treatment-naive and who would have a high placebo response rate.

Future studies of nonpharmacologic therapies should address the relationship between the efficacy seen in single-site trials and what was found in this single large effectiveness trial. Phase II trials of pharmacologic therapies for CFS and fibromyalgia provide an opportunity to innovate with respect both to study design and outcome measures. Improvements seen with various classes of antidepressants in conditions like fibromyalgia and CFS are independent of whether that person had a mood disorder at baseline. Whether or not they respond to a tricyclic drug is not related to whether they are depressed, whether their pain will get better, or whether their fatigue will lessen.

Discussion

Question: The one caveat in presenting the VA trial data is the abysmal compliance in the exercise program. Less than 20 percent of subjects completed the full gamut of exercises.

Dr. Clauw: The compliance rate is abysmal in any study of exercise or CBT. If you get a 50 percent compliance rate in a CBT trial or an exercise trial, you are doing well. It was abysmal, but it was not much different than what is seen in other trials using CBT and exercise. However, the effect was quite a bit less.

Dr. Eldadah: Anecdotally, it has been said that Gulf War syndrome is more likely to occur not in front-line soldiers or special operations soldiers, who are more intimately familiar with trauma and perhaps more likely to be risk-takers, but in support-type personnel. This finding raises the issue of expectation.

Dr. Clauw: That is somewhat true. The most consistent finding from the studies that have looked at Gulf War illness is that reservists were much more likely than active duty military to develop Gulf War illness. Some U.S. studies suggest that women were more likely than men to develop it, although that was not found in a United Kingdom study. Gulf War illness did not selectively occur in people who were in the theater of operations. People who left U.S. soil, even if they were far from the theater of operations, were equally likely to develop this constellation of symptoms and syndromes.

Improvements seen with various classes of antidepressants in conditions like fibromyalgia and CFS are independent of whether that person had a mood disorder at baseline.

Cognitive Behavioral Therapy

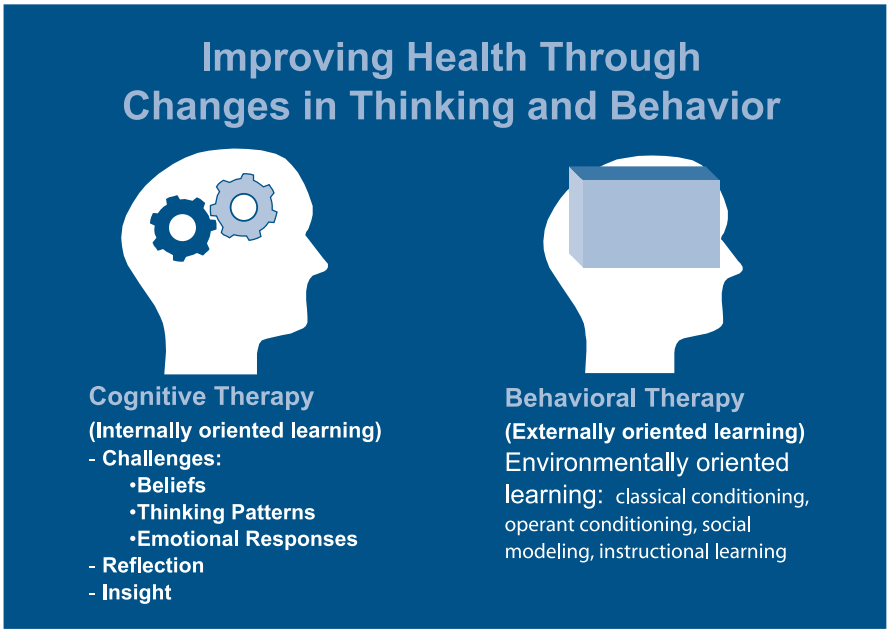
David A. Williams, Ph.D.

Cognitive behavioral therapy (CBT) has been mentioned three times in the last 2 days in relation to studies: Dr. White mentioned that CBT was found to be effective in people with CFS, Dr. Heitkemper stated that CBT was helpful in symptoms but not necessarily with the underlying mechanisms, and Dr. Clauw said that CBT helped increase function in Gulf War veterans but was not as helpful as exercise in alleviating the symptoms of fatigue and pain. Many types of CBT rest on a common set of theories. When evaluating whether CBT is effective, we need to be clear about what type of CBT is being evaluated and for what outcome.

CBT was developed as a therapeutic intervention for the mental illnesses and has strong empirical support for the treatment of depression and anxiety disorders.[1] In the past 15 years, the principles of CBT have been applied successfully to more traditional medical conditions—usually chronic conditions such as cardiovascular disease, cancer, diabetes, asthma, and pain management.[2-4] CBT encompasses the idea that individuals can improve physical health or mental health through changes in thinking and behavior.

CBT is a blending of cognitive therapy and behavioral therapy. Cognitive therapy focuses on the internal mental environment of the individual. Issues commonly addressed are challenges to maladaptive beliefs that individuals may have about their illness or about their health. Cognitive therapy also examines maladaptive automatic thinking patterns and emotional responses to illness. This form of therapy utilizes a great deal of personal reflection and draws upon insight to help individuals gain a clear understanding of their condition and the options that may be available for managing the illnesses they face.

Behavioral therapy focuses on how the external environment affects individuals and on how learned behaviors can contribute to either the management or exacerbation of symptoms.



A number of well-established models of learning are included in behavioral therapies. Classical conditioning, operant conditioning, social modeling, and instructional learning are examples.

With classical conditioning, a neutral stimulus can trigger a physiological response if it is paired with a natural trigger of that physiological response. For example, a bell paired with food will eventually elicit salivation in a dog even when the food ceases to be present. Due to learning, the previously neutral bell takes on the ability to elicit the physiological response. Conditioning has been applied to some degree in medical settings, to help diminish nausea from chemotherapy and to supplement immunosuppressive agents.

Combining the techniques and principles of cognitive and behavioral therapies provides the tenets underlying the principles of cognitive behavioral therapy.

Another form of learning used in behavioral therapies is operant conditioning, which utilizes either positive or negative reinforcement to increase behaviors associated with improved health. For reinforcement to be effective, the desired behavior must occur on its own or be shaped through approximation so that it can begin to be reinforced. Once it occurs, it can be increased through careful monitoring and reinforcement. For example, independent self-care or symptom management can be increased if families are trained to watch for and reinforce efforts of patients to function independently.

Social modeling is based on the idea that an individual does not necessarily have to perform a behavior in order to learn it. Individuals can observe other people performing or behaving and can learn how to mimic those behaviors even though those behaviors have never been rehearsed. Examples include improving one's own functioning by observing how others in a support group have solved their barriers to improved functioning.

Instructional learning encompasses the idea that behaviors can be communicated verbally or through written media; the individual who is supposed to perform the behavior may never observe the actual behavior. Instructional learning is a common form of therapeutics (that is, an educational intervention) but there is no guarantee that someone who understands the concepts concerning a behavior will ever actually perform that behavior.

Combining the techniques and principles of cognitive and behavioral therapies provides the tenets underlying the principles of CBT. Given that many different principles constitute CBT, many versions of this form of therapy can emerge.[5]

How might CBT be used in CFS? Versions of CBT focus on sleep, physical functioning, mood, memory, stress, and tension. When used rationally, the choice of CBT techniques should target a specific problem. Thus, when a form of CBT is chosen, it should follow some hypothesis about the underlying mechanism driving the illness. In the case of CFS, this presents a problem because the underlying mechanisms to be targeted are not yet known. Many of the trials that have used CBT have tried to hypothesize what mechanisms might be at play. Friedberg and Jason recently published a review paper in which they proposed a number of different models for understanding CFS.[6] These models include an immune deficit model, a sleep disturbance model, a neuroendocrine abnormality model, a predisposing personality model, and a symptom avoidance model. Because of the lack of consensus regarding the underlying mechanisms of CFS, CBT interventions to date are directed at managing overt symptoms.

Most currently available forms of CBT for CFS have been borrowed from successful applications in other illnesses. Because there is no unified model, many forms of CBT are tried with the hope that one of them will make a difference. To date, little evidence of harm from this approach has been noted and there is some evidence of benefit in some people.

One strategy that might be used is to teach the relaxation response. The relaxation response has been used extensively in the field of chronic pain and insomnia. A *JAMA* paper in 1996 followed an NIH technology assessment panel that suggested that relaxation is a useful nonpharmacological intervention for pain and insomnia, both of which have relevance to CFS.[7] The relaxation response can be taught in a variety of ways: progressive muscle relaxation, visual imagery, meditation, biofeedback, and yoga. To date, no one method is superior to others, but studies suggest that effective use of the relaxation response depends on the individual's preference. Thus, tailoring the intervention to the individual

allows the individual to use the method he or she finds most appropriate for achieving the relaxation response.

Another behavioral strategy is sleep hygiene, which is based on the idea that an individual can make behavioral changes to bring about improvement in sleep.[8] Timing strategies are an example. Individuals are encouraged to maintain a regular waking and retiring time so that the body can learn a regular pattern for sleep. Patients are encouraged not to stay in bed if they cannot fall asleep, and to get out of bed if they do not fall asleep within 15 minutes. This helps to prevent the bed from being associated with struggle and discomfort. Thermal tips include taking a warm bath or exercising several hours before going to bed and maintaining an environment conducive to sleep, which is often a problem expressed by individuals with CFS. Substances like caffeine and nicotine should be avoided as should stimulating television or reading material.

Stress management and assertiveness training represent another form of CBT.[9] Over the course of time, the types of stressors that humans face have shifted. Historically, the stressors faced by humans were physical in nature and short lived. In the case of wild animals, hostile weather, plagues, and food shortages, people either survived those stressors or perished. After about 1900, threats from the physical world diminished but threats from the social world greatly increased. In modern times, many stressors are social stressors involving the work place, family life, litigiousness, and government agencies. These stressors do not kill us and therefore can exist and wear on us for long periods of time. Due to the interpersonal nature of many modern stressors, assertiveness training can help individuals communicate more effectively and form win–win relationships with others. In our work, assertiveness training has turned out to be one of the most highly endorsed and satisfying skills taught to patients with fibromyalgia. This form of stress management has been very useful and is considered relevant to daily functioning and quality of life, if not to direct symptom expression.

Cognitive reappraisal and structured problem-solving draws heavily from skills used in cognitive therapy.[10-12] While it may still be debated whether emotions drive thinking or thinking drives emotions, for the purposes of CBT it is useful to assume that people learn automatic thoughts in response to certain situations; these automatic thoughts then drive emotional responses. Inasmuch as these emotions are negative, symptoms can be made worse. The goal of cognitive reappraisal is to intervene at the level of the automatic thought and alter the thinking process so that a fresh and potentially more adaptive set of emotional responses can emerge for common situations. The goal with this form of therapy is not to produce unrealistic bliss, but to challenge intense negative automatic thinking that might be blinding the individual to new and potentially adaptive alternatives.

Problem-solving is a useful form of therapy for individuals with chronic illnesses who tend to have more complex problems than the average individual. It is often helpful to break these problems into solvable parts. A number of five- or six-step problem-solving processes exist in which individuals learn to break large problems down into more manageable parts.[13-14]

Because of the lack of consensus regarding the underlying mechanisms of CFS, CBT interventions to date are directed at managing overt symptoms.

Another behavioral strategy is graded activation, also known as time-based pacing or activity-rest scheduling.[15-17] Individuals with a chronic condition like fibromyalgia or CFS will often engage in a destructive pattern of over-activity followed by symptom flare-ups and the need for prolonged rest and recovery. This latter period is often accompanied by feelings of diminished self-worth and frustration due to diminished productivity. When feeling better, individuals again tend to overdo in an attempt to "catch-up"; the result is often another flare-up, producing a cycle that is nonproductive. When patients monitor their activity and rest periods over time, they discover that this cyclical pattern of activity results in shorter periods of productivity and longer periods of recovery equaling less productive time during the day.

Many people think that task-based pacing is the solution to increasing function, but it is not. Task-based pacing, which involves pacing one's activities based on completion of a task, can still result in overdoing; time-based pacing is more helpful. In time-based pacing an individual is active for a specific amount of time, which is then followed by a specific amount of rest, followed by another specific amount of activity based on time, and

followed by timed rest. This form of pacing is admittedly unnatural, and people have difficulty initiating this type of pacing; however, once they do, the number of flare-ups decreases and functional time rises. The goal of this strategy is to find the safe amount of time an individual can engage in a specific activity, which is where the individual must start pacing. If the individual stops within this safe amount of time and begins to rest, then the recovery time will be much briefer since recovery will be tied to a safe amount of activity rather than to a flare-up. It might take a little longer to perform a given task by stopping and resting before it is done, but prevention of a flare-up means that other tasks can also be accomplished with less pain or fatigue.

It may be difficult to initiate the use of time-based pacing in the workplace; therefore, in therapy, individuals often start by pacing pleasant activities.[18] Given that individuals with chronic illnesses like fibromyalgia or CFS list the lack of pleasant activities as a problem, pleasant activity "scheduling" is another CBT skill that can be used to help manage symptoms and improve the quality of life of individuals with these conditions.

What approaches work best? About 30 clinical studies have investigated CBT for fibromyalgia, but few can be considered well-conducted, randomized control trials. Few studies have used the same combination of CBT skills, making comparison difficult among trials. The CBT literature for CFS is somewhat smaller and suffers from a similar lack of uniformity; however, several CFS studies that followed a randomized control design hold promise. In one such study, 60 patients with CFS were randomly divided into two groups—a cognitive behavioral group and a standard care group. The types of CBT used were problem solving, graded activation, and cognitive restructuring, delivered in 16 sessions. A 12-month outcome looked at improvements in physical functioning. Results showed that 73 percent of the subjects receiving CBT improved in physical functioning compared to only 27 percent of those receiving standard care. In the CBT group, improvement was about 175 percent improvement over 12 months, and the number needed to treat was two in order to get one return to normal functioning.[19]

Another study compared graded activation and cognitive restructuring CBT with progressive muscle relaxation in 13 sessions over 4 to 6 months. Improvements in physical functioning, fatigue, mood, and global improvement were the outcomes. Results showed that 70 percent of the CBT group improved in physical functioning compared to 19 percent of the relaxation-only group. In the CBT group, improvement was at 270 percent over 6 months, and the number needed to treat was two in order to get one return to normal functioning.[15]

Another study found that CBT was better than a support group or a natural course. A total of 93 individuals were involved, using the CDC criteria, and the groups were CBT, guided support group, and natural course. The types of CBT used were graded activation, cognitive restructuring, and activity scheduling. This study comprised 13 sessions over 4 to 6 months, and the outcomes were physical functioning, fatigue, and global patient improvement. Results showed that physical functioning was improved and fatigue was lessened in the CBT group, and the support group and the natural course group had about equal outcomes. After 14 months in the CBT group, 49 percent of subjects showed meaningful clinical improvement in physical functioning, 35 percent improved for fatigue, and 50 percent improved on the global impression of improvement.[20]

About 30 clinical studies have investigated CBT for fibromyalgia, but few can be considered well-conducted, randomized control trials.

One of the earlier studies was somewhat negative, concluding that CBT was no different than immune therapy. The groups were CBT, immune therapy, a combination of CBT and immune therapy, and standard care. The form of CBT combined exercise and cognitive restructuring, in only six 60-minute sessions conducted over 2 weeks. The outcomes were global health, physical functioning, and psychological functioning. Improvements were seen, but they were not specific to any single intervention.[21]

Summarizing the studies to date, the strongest evidence is for the use of graded activation and cognitive restructuring in CFS. There is moderate support for problem-solving, activity scheduling, and relaxation. The least support (because too few studies have been conducted) is for use of sleep hygiene, assertiveness, and a general stress

management approach. Areas of additional research needed include: explorations of CBT in mild forms of CFS, and effectiveness of specific forms of CBT for individuals with severe forms of CFS who may be unable to attend CBT sessions due to the severity of their disease. Group CBT is understudied; most studies have used a more traditional one-on-one format. CBT also should be studied in generalized practice, as opposed to specialized practice. Most of the well-conducted studies so far have used an efficacy design in which a single site has been used. The exception is the Prins et al. study that looked at 13 therapists of varying quality; this study showed lower effectiveness than in other studies that used a single site and specialized clinics.

Future studies should further explore the use of CBT techniques to manage symptoms, improve quality of life, and eventually address underlying mechanisms of CFS as consensus builds on this issue. While CBT to date cannot be considered a curative intervention, it does appear to dramatically improve the condition in a subset of individuals possessing the diagnoses of CFS or fibromyalgia.

Discussion

Question: What is the effect of CBT on beliefs such as interpersonal threats and anxiety, and also locus of control?

Dr. Williams: Beliefs such as locus of control are likely to be important in determining outcomes. We have a study in development that will look at locus of control, so I will hopefully be able to answer this question more specifically in a year or so. There are some studies in FM and other chronic pain conditions that have successfully enhanced internal locus of control with CBT. What we think happens is this: individuals who successfully engage in activities, such as pacing or graded activation, learn something about themselves (for example, they can manage their condition while improving function); those new achievements or new abilities to function make it difficult to retain a belief in one's inability to function. Thus, changes in behavior support changes in beliefs. Simply telling folks to change how they think is not likely to be effective, but it is hard to argue with your own personal experience that you are indeed doing better. In our attempts to change beliefs, we usually try to set the stage for cognitive change by providing individuals with actual experiences where they are doing better.

Question: What about changing a belief such as disability? The person believes he/she is disabled. Was that one of the biggest potential problems of the Gulf War *JAMA* article?[22]

Dr. Williams: Disability was a major predictor of outcome. I do not know how we would try to change a belief in disability, given that it is probably heavily reinforced by so many environmental factors. As a therapist you would need to have control over much of the environment to make a meaningful change in that belief, and this is not likely. It might be possible to have people work to change their behaviors in spite of the belief in disability, especially if they are seeing an improvement in functioning. Compliance or adherence, however, is likely to be problematic under these conditions.

Question: Regarding the pacing point, are the reasons behind that known? For instance, is it realistic compensation across people for the limitations from their condition, or is it certain personality types?

Dr. Williams: The problem with pacing is that when individuals are inactive they may have an expectation of what they "should" be able to do—this may be based on a previous history of being highly functional. Internally, there may be a drive to try to recapture the lifestyle before illness and its associated level of functioning. As people try to live as they used to, they may push their bodies to do things that it cannot do at the moment, causing an increase of symptoms and then a longer period of time during which function is markedly reduced. On a cognitive basis, there is a real disconnect between what the person wants to do (that is, what he or she thinks is possible) and what is actually possible. The motivation to feel unencumbered by illness (even for a short while) may override the knowledge that there will be a price to be paid for overdoing.

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Summarizing the studies to date, the strongest evidence is for the use of graded activation and cognitive restructuring in CFS.

Challenges in the Development of Acute and/or Chronic Pain Therapies

Lee Simon, M.D.

The Food and Drug Administration (FDA) is heavily invested in trying to understand the issues associated with how to adjudicate outcomes in acute and chronic pain.

Chronic pain is a subjective event. Transference from an established and acute pain syndrome to chronic pain is different for each person, and significant subjective issues are associated with the outcome. The FDA is interested in the implications of that transference and the syndromes associated with chronic pain. It is likely that the exact cause of conversion to chronic pain is not the same in each circumstance, and therefore outcomes are more challenging. Chronic pain cannot be addressed without addressing acute pain.

It is only a belief system, and is not true, that the FDA prevents the development of drugs by taking "forever" to approve them. The amount of time it takes to develop a new therapeutic intervention is based on conducting the clinical trials that provide the evidence that the new therapeutic product actually works. Much of the FDA's work translates to the end product which, for physicians or patients, is the therapeutic product.

The end product for the FDA is the label associated with the drug. The label has legal and regulatory uses, but the real purpose is to inform prescribers and patients about the documented benefits and risks of a product. The only place to obtain unbiased information about a product is the FDA label.

The components of the label are useful to practitioners and patients—results of clinical studies, how to use a particular agent, and what the agent is indicated for. The FDA does not regulate medical care, but the indications do imply that the agent has been studied. Contraindications to use, warnings, precautions, adverse reactions, and dosage administration are included in the FDA label. FDA labels will change shortly; the indications and usage will be placed first so people can open up the label and use it in an effective way instead of having to read through a lot of verbiage.

The FDA evaluates the risks and benefits in the context of a population. It is hoped that people become educated through the label and that the label provides the learned intermediary with the ability to evaluate the benefits and risks for a patient they advise. A patient may also be able to understand the information provided and to evaluate the benefits and risks in terms of their own values. Societal values are part of making a decision about therapeutic intervention. For example, thalidomide has just been approved in a restricted way for use in the United States; the societal machinations were significant prior to deciding that this agent deserves to be on the market for specific uses.

Transference from an established and acute pain syndrome to chronic pain is different for each person, and significant subjective issues are associated with the outcome.

Acute pain is a self-limiting condition that informs how a drug for pain relief should be used, studied, and labeled. Off-label use can result in serious adverse events, and perhaps even death. However, some therapeutic interventions warrant taking a certain risk relative to certain safety signals, depending on what the drugs are designed to do. If a patient is at risk because of an underlying disease and substantial benefit could accrue with

a particular therapeutic product, the potential for benefit may warrant taking the associated risk, even though that risk may include a risk of death. Oral analgesics are used primarily in outpatient settings and parenteral analgesics are used for inpatient settings. While the nature of pain is subjective for each individual, the FDA is tasked with determining the meaning of pain relief in large population studies and whether a drug destined to be used acutely provides acute relief. For one person, acute relief might be defined as within 10 seconds; another person might define it as within 2 hours. The FDA standard is that an acute pain drug must have an onset within 60 minutes.

The FDA is interested in time to onset, peak effect, effect size, and duration. Patients who take pain drugs would like to know that, for example, 80 percent of the subjects who were given the drug got pain relief within 1 hour, and the drug produced an 80 percent pain relief that lasted for 12 hours in 70 percent of the subjects. Unfortunately, that specificity does not yet exist within the labeling environment at the FDA.

Nonsteroidal anti-inflammatory drugs are extraordinarily effective for dental pain; morphine is basically ineffective compared to 400 milligrams of ibuprofen in that model. However, in other models such as bunionectomy, 400 milligrams of ibuprofen does not have any effect and a patient-controlled analgesia pump is useful.

In single-dose studies of post-surgical abdominal, gynecological, and orthopedic pain, 940 patients were given doses of one or two tablets. Drug X produced greater efficacy than placebo, and no advantage was demonstrated for the two-tablet dose. For short-term (generally less than 10 days) management of acute pain, the recommended dose of drug X is one tablet every 4 to 6 hours as necessary, and dosage should not exceed five tablets in a 24-hour period.

A more informative label might contain the following attributes:

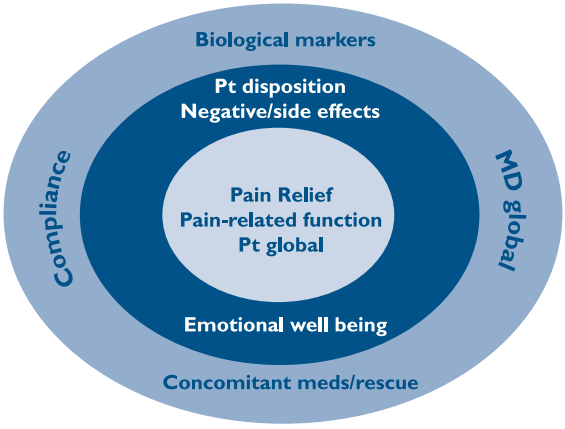
- "Initial pain benefit beginning at X minutes after the first dose in a percentage of patients." This information would note the different models of pain, which would give the provider a sense of which drug and how much to prescribe.
- "Duration of pain relief is for X hours in a percentage of patients in Y model, and a second dose was required Z number of hours after the first dose in some patients in that model."

The *Merck Manual* (17th edition, 2002) defines chronic pain as pain persisting for greater than 1 month beyond the acute injury, persistent or recurring pain for at least 3 months, and pain if expected to continue or progress perhaps (but not necessarily) associated with subsequent and ongoing tissue injury. Chronic pain has no adaptive role, and depression may follow as a consequence. Expectations of pain relief have to be tempered by how long patients will survive with their illness, so the same expectations cannot be held for cancer pain models and noncancer pain models, for example. Studying cancer pain models may be limited to 6 weeks of experience, whereas in a chronic pain scenario, 6 weeks of experience is not particularly long.

Fibromyalgia is a real challenge. It is unknown whether the pain is fibromyalgia or whether pain is one manifestation of the disease called fibromyalgia. Many data suggest that this might be a neuroendocrinologic disorder.

There are many different ways to ask whether or not pain gets better. Objective measures exist, but there is always a subjective component when measuring pain. In certain circumstances, other data are needed to support the evidence that pain has changed. To have a change in pain, there must be some evidence of a minimally clinically important difference, but how to determine that difference is subjective.

Pain Domains?



In osteoarthritis, pain and function are highly correlated; in other scenarios, such as fibromyalgia, they are not. The FDA is interested in understanding the central component of what it means for the patient to get better because buried within that concept is a sense of the patient's health-related quality of life. It is also possible that treating pain will make the patient worse; measuring the level of pain is difficult but is considered a critical outcome by the FDA. For example, in osteoarthritis, when the knee is targeted, it is essential to ensure that other joints in the body do not worsen. In the context of rheumatoid arthritis, health-related quality-of-life measures are difficult to assess in patients taking Methotrexate, because Methotrexate nauseates the patient when it is first taken. Comparator drugs do better on a quality-of-life scale because they do not make patients nauseous.

Most clinical studies show that the majority of physicians have no clue how to measure outcomes. Reporting that a patient is better is more related to ego than it is related to actual improvement.

Metrics for measurement of pain include health-related quality-of-life measures, the utility and use of rescue medications, the economics of the intervention, the presence of organ damage because of both the intervention and the causality of pain, the issue of suffering, and the issue of adverse events and what transpires because of the intervention.

Osteoarthritis can be used as an example of these domains. The osteoarthritis domains enumerated by the Outcome Measures in Rheumatoid Arthritis Clinical Trials (OMERACT, an international organization related to the World Health Organization) are similar to those adopted by the FDA for its primary outcomes: pain, function, and "patient global" for the signs and symptoms of osteoarthritis. (The domains are not related to the pain of osteoarthritis; that indication does not exist.) The function component of this outcome is defined by the Western Ontario and McMaster University Osteoarthritis Index as the result of asking 24 questions about function, 2 about stiffness, and 5 about pain. A claim of improvement in osteoarthritis is a claim of improvement in the signs and symptoms, measured by answers to questions related to pain, patient global, and the self-administered questionnaire for function. A proposed trial would be 3 months in length but greater than 1 year after developing a structural plane. Other areas to be evaluated during this trial will include other joints, rescue medications, the development of osteophytes if a structural plane is present, and the utility and use of devices. Pain and function will be analyzed separately, allowing for a better understanding of the disease state versus the generalized pain state. In rheumatoid arthritis, the FDA uses a responder index called the American College of Rheumatology/World Health Organization ILAR response index, which measures the tender and swollen joints, and then measures three of the following five: a scale for pain, patient global, physician global, health assessment questionnaire, and/or a sedimentation rate. The multimodality of rheumatoid arthritis, which also has pain as a component, might make it more reflective of fibromyalgia than would be a pain function global for a local disease such as osteoarthritis.

To have a change in pain, there must be some evidence of a minimally clinically important difference, but how to determine that difference is subjective.

No product in the world, including water, is totally safe and totally effective. Determining safety is made even more complex because society has different ideas about what is safe compared with what an individual might believe. Measuring those differences and those values is difficult; Europeans seem able to measure these constructs more effectively than researchers and regulators in the United States. As the new world of molecular pharmacology evolves, society will have to pay more attention to deciding some of these issues.

The goal is to create and recognize which drugs will affect patient K differently than M and differently than F. The subjective nature of pain will make a difference in how people experience the end result. It will be critical to develop a concept of minimally clinically important differences that society and individual patients can accept. This concept will remove the concern about numbers of patients in a trial, what an effect size might mean, and what the P-value means. The FDA will not be able to accomplish that alone; the implications for societal expectations are

significant when considering what a $1 billion drug might require and deliver, especially if the per-patient cost of such a drug is $10,000 a year.

The ideal characteristics of a pain metric include that it should be easy to understand by patients and clinicians, because patients and clinicians conduct and participate in the studies and provide the answers to research questions. Truth in labeling requires description of the clinical studies to the providers and the subjects so they understand the implications of the intervention. Measures also must be applicable across studies to allow an understanding of the effect of a particular therapeutic. A pain metric should define a clinically meaningful result, so it can be deemed a useful addition to pain control. "Clinically meaningful" must include a definition of the patient actually functioning better in society as a result of the intervention. It has to be valid in a variety of painful conditions, and it has to be achievable with the current medications.

Rescue medications are used when a patient fails a therapeutic intervention trial. There may be synergism with rescue medications, and trials should be designed to answer that question.

Another possible approach is in the context of a mechanistic claim. Does a therapeutic product prevent wind-up? Does it alter N-methyl-D-aspartate activity? Does it reduce prostaglandin levels such as in Cox 2 upregulation in the thalamus? Does it treat acute neuropathic pain (which is different from other kinds of pain)? Does it reduce nonsteroidal and opioid use? Mechanistic claims are popular for many industrial sponsors because they are easily measured.

At this stage for pain relief related to fibromyalgia, the important measures are known and can be incorporated into a clinical trial: improvement in measurable pain, improvement in patient function, and improvement in "patient global." Other measures might be important as early markers of change that might predict, but are not anchored to, clinical improvement: a change in the hypothalamic-pituitary-adrenal axis or a change in the neuroendocrine system, such as a hormonal change.

The FDA believes that trial length for fibromyalgia should be 3 to 6 months, with at least a year of exposure for safety signals as defined by the International Harmonization Guidelines. Whether 6 or 10 weeks is long enough is being debated regularly. Other questions being asked by the FDA relating to fibromyalgia include:

- Should a patient have decreased symptoms, and for how long with and without therapy?
- Are fewer tender points, either by dolorimeter or otherwise, enough to suggest decreased pain?
- Is the fibromyalgia questionnaire, the FIQ, an adequate measure of function? Is it improvement in the pain of fibromyalgia or improvement in the disease?
- Would a cure be required, and what would be necessary to be considered a cure?

At this stage for pain relief related to fibromyalgia, the important measures are known and can be incorporated into a clinical trial: improvement in measurable pain, improvement in patient function, and improvement in "patient global."

Instead of improvement being measured by a lessening of the pain of fibromyalgia in this multisystemic disorder, improvement in fibromyalgia overall will be the standard, which will include the concept of improvement in pain.

The FDA web site provides information about all of these areas: www.fda.gov. Information about the Division of Analgesic, Anti-inflammatory, and Ophthalmic Drug Products can be accessed from the FDA web site as well.

The current world of drugs is one of action and reaction, with drug exposure and drug experience. Drug exposure issues are important, and have much to do with informed consent issues in clinical trials and post-marketing indications. Drug experience is an issue of benefit, weighing the risks such as adverse events against the patient's feeling and functioning better and improvement in the underlying process or disease. The risks and benefits must be defined accurately and must be evidence-based so the appropriate decisions can be made by and for patients.

Discussion

Dr. Krueger: Is there any movement within the FDA to consider the cost of therapy as one of the criteria that factor into drug approval?

Dr. Simon: Presently, the only places in the world where a pharmacoeconomic outcome is required for approval are Canada and Australia, although AMIA and the European community are moving in that direction. It is likely that economics will play an increasing role, not in approval but in effectiveness measures. The FDA is legislated by Congress only to deal with efficacy, not effectiveness. Until Congress decides to change that issue, we do not have any legal authority to apply the issue of economics as an outcome.

Dr. Crofford: Have you given any thought to what kinds of outcomes or indices may be useful for a condition like CFS, where fatigue is even more subjective and more difficult to understand than pain?

Dr. Simon: At our last OMERACT meeting 2 years ago, we invited patients for the first time to attend. About 35 patients attended; they were not all Australians. The patients informed us that physicians are interested in the context of rheumatoid arthritis and X-rays and the researchers are interested in the concept of function in osteoarthritis, but patients are really interested in fatigue. When you get doctors together, fatigue always gets thrown out because it is difficult to measure. We are going to have to grapple with fatigue as an outcome in all the inflammatory diseases. The reason is that patients care about fatigue and, fundamentally, we are developing drugs to make patients better—and patients tell us they want to be better in that way.

Dr. Crofford: In thinking about developing outcome measures for fibromyalgia, patients may tell you that fatigue is their most important symptom in even higher proportions than would osteoarthritis and rheumatoid arthritis patients. Is there any thought by the FDA that fatigue might be reflective of function?

Dr. Simon: We have built into our discussions that the patient global included that kind of issue. A patient global response has to be dependent on the kind of question asked. Fatigue will be one of the major topics of the workshop co-sponsored by the FDA and the NIH scheduled for the fall of 2003, so fatigue will receive a tremendous amount of investigation and interest, and will be elevated into the real world of being part of the responder index.

Dr. Zubieta: We are trying to better understand pathophysiologies of illness. We develop new technologies such as functional MRI, and we are able to measure receptors in the human brain. From the FDA's perspective, to what degree would it be advantageous for drug companies to use these types of measurements to define how well a drug is saved by a receptor site, or how well it correlates to a particular outcome measure?

Dr. Simon: From the regulatory perspective, we would be very interested in working with any sponsor to look at PK/PD understanding, predicting dose better, and all other early phenomena. To be able to use binding to a receptor as a clinical outcome or as a surrogate for a clinical outcome is not likely to be accomplished within my lifetime. We would like to foster that kind of investigation, which we think can be useful as an early marker to understand how to predict pharmacodynamics. Perhaps someday it may be useful in other ways, even in approvals, but not for a long time.

Session Six: Optimal Directions for Research of the CNS in CFS

Co-Chairs: Leslie J. Crofford, M.D., and Dedra Buchwald, M.D.

Summary of Discussion Sessions

The themes of the discussions from this meeting can be grouped into two major categories:

1. *Who are chronic fatigue syndrome patients?* Some people lump everybody into one setting; some people split CFS patients into different categories; and others categorize CFS patients as somewhere between the two extremes. "Lumping" and "splitting" are concepts of categorization with which the field will have to grapple. Many "splitters" think that, in order to attain a uniform standard patient population, patient populations should be defined according to some biomarker or measurable phenotype that can be demonstrated, rather than symptoms that are sometimes difficult to interpret. Who should be studied when seeking to understand this disease: Populations? Clinical cohorts? Cross-sectional cohorts? Inception cohorts in the case of an acute illness? Patients or families? All of the above or none of the above?
2. *What should be studied?* The ultimate outcome from this meeting is the understanding that the brain is significantly more complicated than the liver and requires more complicated measuring methods. Studying the brain requires precision in deciding what to study. For example, fatigue is a symptom of thyroid disease, but it is a symptom of both hypothyroidism and hyperthyroidism. Certain CNS systems work in the same way. Symptoms related to the CNS are plastic, and dynamic variability is common. Measurements of liver function tests, for instance, usually produce results that are reproducible; the brain is more complex, and it is necessary to control for quite a few more variables than is necesary with more static systems.

The original question of this meeting was: Is the central nervous system a valid topic for study in patients with CFS and related disorders? The consensus among most of the presenters appears to be: yes, it is a valid topic. For building models, the current question is why, and at what node, is the CNS an important study topic. For future directions, the key question is how to use the information presented in this concept that the CNS may be involved at single or multiple nodes along the development of symptoms that are related to CFS and other disorders.

The CNS Hypothesis in the Design of Future Studies

A model must incorporate the variability and clinical manifestations of CFS, including psychological distress, a variety of laboratory findings, the physical exam, and the presence or absence of overlapping conditions. A number of specific symptoms appear to be very important in CFS, for example, exercise intolerance, or post-exertional fatigue. Female predominance has come up in discussion of irritable bowel syndrome, temporomandibular disease, fibromyalgia, and CFS. Familial clustering occurs in fibromyalgia, CFS, and other fatiguing conditions. Another element that a useful model of CFS must address is the altered perception or belief state for some of the crucial symptoms of CFS, such as exercise and cognition. Acute onset reported by most patients, although not all patients, must be addressed. It may be crucial to understand what kinds of illnesses CFS patients do not get, because that knowledge can also be a key to defining CFS and to developing models to understand CFS.

Methodological and Other Issues to Resolve

- CFS studies must be longitudinal and conducted in multiple centers with sufficient

subject numbers for statistical power and must be of sufficient duration to account for cycles of remittance and relapse.

- Methodological limitations, such as sampling time, specimen shipping conditions, processing methods and preservation, must also be standardized.
- Useful phenotypes for the symptoms of CFS must be developed and defined so they can be measured accurately.
- An animal model for CFS must be developed.
- Researchers should take advantage of the newer scientific methods and techniques that can be applied to provide immediate insights into the symptoms of CFS. Use of these approaches will ensure that the projects undertaken are interdisciplinary.
- Unexplained clinical parameters include variability in clinical manifestations, specific symptoms (for example, exercise intolerance), female predominance, altered perception/belief, correlates of acute onset, and exposures—medical histories remain vitally important as a key element in how to explain chronic diseases.

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Trans-NIH Working Group for Research on Chronic Fatigue Syndrome

ORWH, Chair

Dr. Eleanor Hanna
Associate Director for
Special Projects and Centers
Office of Research on Women's Health
Office of the Director
National Institutes of Health
Executive Plaza South
6120 Executive Blvd., Room 150A
Bethesda, MD 20892-7116
Phone: 301-435-1573
Fax: 301-402-0005
E-mail: eh43q@nih.gov

CSR

Dr. J. Terrell Hoffeld
Center for Scientific Review
National Institutes of Health
Rockledge 2 Building, Room 4116
6701 Rockledge Drive, MSC 7816
Bethesda, MD 20892-7816 (postal)
Bethesda, MD 20892-1813 (courier)
Phone: 301-435-1781
Fax: 301-480-2644
E-mail: th88q@nih.gov

NCCAM

Dr. Richard Nahin
Senior Advisor for Scientific Coordination
and Research
National Center for Complementary and
Alternative Medicine
National Institutes of Health
9000 Rockville Pike, MSC 2182
Bethesda, MD 20892-2182
Phone: 301-496-7801
Fax: 301-435-6549
E-mail: nahinr@mail.nih.gov

NCRR

Dr. John D. Harding
Health Scientist Administrator
Division of Comparative Medicine
National Center for Research Resources
National Institutes of Health
6701 Democracy Boulevard
Bethesda, MD 20892-4874
(use "20817" for express service)
Phone: 301-435-0776
Fax: 301-480-3819
E-mail: hardingj@ncrr.nih.gov

NHLBI

Dr. Carl E. Hunt
Director
National Center on Sleep Disorders Research
National Heart, Lung, and Blood Institute
National Institutes of Health
Rockledge 2, Room 10-038
6701 Rockledge Drive, MSC 7920
Bethesda, MD 20892-7920
Phone: 301-435-0199
Fax: 301-480-3451
E-mail: HuntC@nhlbi.nih.gov

Dr. Stephen S. Goldman
Health Science Administrator
Division of Heart and Vascular Diseases
National Heart, Lung, and Blood Institute
National Institutes of Health
6701 Rockledge Drive, Suite 10193
MSC 7956
Bethesda, MD 20892-7956
Phone: 301-435-0560
Fax: 301-480-2858
E-mail: goldmans@nhlbi.nih.gov

NIAA

Dr. Denise Russo, Ph.D.
Division of Metabolism and Health Effects
National Institute on Alcohol Abuse and Alcoholism
National Institutes of Health
5635 Fishers Lane, Room 2037
Bethesda, MD 20892-9304
Phone: 301-402-9403
Fax: 301-594-0673
E-mail: drusso@mail.nih.gov

NIAID

Dr. David M. Morens
Program Officer
National Institute of Allergy and Infectious Diseases
National Institutes of Health
6610 Rockledge Drive, Room 4097
Bethesda, MD 20892-7630
Phone: 301-496-7453
Fax: 301-480-1594
E-mail: DMORENS@niaid.nih.gov

NIAMS

Dr. Deborah N. Ader
Program Director
Behavioral and Prevention Research
National Institute of Arthritis and Musculoskeletal and Skin Diseases
National Institutes of Health
Building 45, Room 5AS-19H
45 Center Drive, MSC 6500
Bethesda, MD 20892
Phone: 301-594-5032
Fax: 301-480-4543
E-mail: aderd@mail.nih.gov

Dr. Susanna Serrate-Sztein
Director
Rheumatic Diseases Branch
National Institute of Arthritis and Musculoskeletal and Skin Diseases
National Institutes of Health
Building 45, Room 5AS-37J
45 Center Drive, MSC 6500
Bethesda, MD 20892
Phone: 301-594-5032
Fax: 301-480-4543
E-mail: ss86e@nih.gov

NICHD

Dr. Lynne M. Haverkos
Director
Behavioral Pediatrics and Health Promotion Research Program
National Institute of Child Health and Human Development
National Institutes of Health
6100 Executive Boulevard, MSC 7510
Room 4B05B
Bethesda, MD 20892-7510
Phone: 301-435-6881
Fax: 301-480-7773
E-mail: haverkol@mail.nih.gov

Dr. Louis Quatrano
Program Director
National Center for Medical Rehabilitation Research
National Institute of Child Health and Human Development
National Institutes of Health
6100 Executive Boulevard, Room 2A01
Bethesda, MD 20892
Phone: 301-402-4221
Fax: 301-496-0832
E-mail: q2n@nih.gov

NIEHS

Dr. Anne P. Sassaman
Director
Division of Extramural Research and Training
National Institute of Environmental Health Sciences
National Institutes of Health
MD EC-20
P.O. Box 12233
Research Triangle Park, NC 27709
Phone: 919-541-7723
Fax: 919-541-2843
E-mail: sassaman@niehs.nih.gov

Dr. Annette Kirshner
Program Administrator
National Institute of Environmental Health Sciences
MD EC-23
P.O. Box 12233
Research Triangle Park, NC 27709
Phone: 919-541-0488
Fax: 919-541-5064
E-mail: ak27b@nih.gov

NIMH

Dr. Peter Muehrer
Chief
Health and Behavioral Science Research Branch
National Institute of Mental Health
National Institutes of Health
NIH Neuroscience Center, Room 6189
6001 Executive Boulevard, MSC 9615
Bethesda, MD 20892-9615
Phone: 301-443-4708
Fax: 301-480-4415
E-mail: pmuehrer@mail.nih.gov

Dr. Matthew V. Rudorfer
Associate Director for Treatment Research, DSIR
National Institute of Mental Health
National Institutes of Health
NIH Neuroscience Center, Room 7163
6001 Executive Boulevard, MSC 7163
Bethesda, MD 20892-9635
Phone: 301-443-1111
Fax: 301-443-4045
E-mail: mrudorfer@nih.gov

NINDS

Dr. Linda Porter
Program Director
National Institute of Neurological Disorders and Stroke
National Institutes of Health
NSC, Room 2113
6001 Executive Boulevard
Rockville, MD 20892
Phone: 301-496-9964
Fax: 301-402-2060
E-mail: porterl@ninds.nih.gov

NINR

Dr. Martha L. Hare
Program Director
Office of Extramural Research Programs
National Institute for Nursing Research
National Institutes of Health
One Democracy Plaza, Room 710
6701 Democracy Boulevard
Bethesda, MD 20892-4870
Phone: 301-451-3874
Fax: 301-480-8260
E-mail: harem@mail.nih.gov

OBSSR

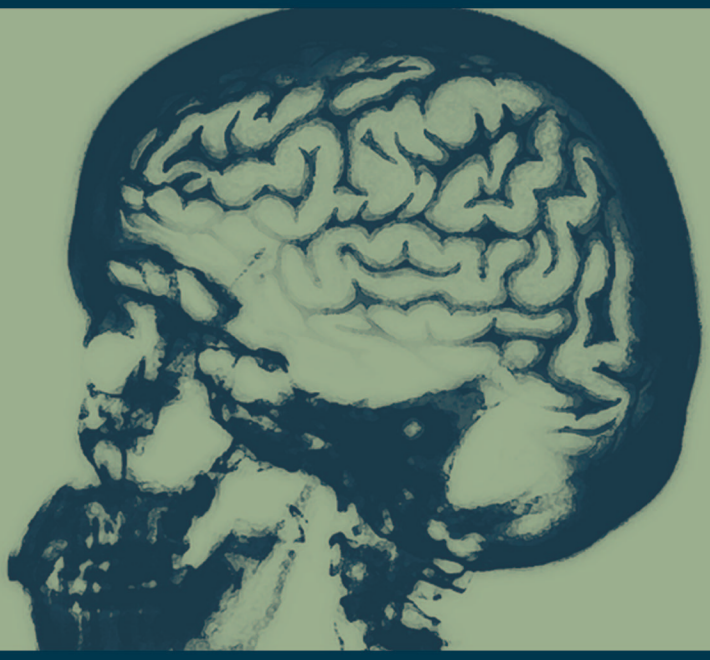
Dr. Barbara DeVinney
Office of the Director
Office of Behavioral and Social Sciences Research
National Institutes of Health
Building 1, Room 256
1 Center Drive
Bethesda, MD 20892
Phone: 301-402-3930
Fax: 301-402-7555
E-mail: Devinneb@od.nih.gov

ODS

Dr. Rebecca B. Costello
Deputy Director
Office of Dietary Supplements
National Institutes of Health
6100 Executive Boulevard
Room 3B01, MSC 7517
Bethesda, MD 20892-7517
Phone: 301-435-2920
Fax: 301-480-1845
E-mail: CostellB@od.nih.gov

ORWH

Ms. Joyce Rudick
Director of Programs and Management
Office of Research on Women's Health
Office of the Director
National Institutes of Health
Building 1, Room 201
1 Center Drive
Bethesda, MD 20892
Phone: 301-402-1770
Fax: 301-402-1798
E-mail: rudickj@od.nih.gov



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