INTEGRATIVE THERAPIES FOR DEPRESSION

Redefining Models for Assessment, Treatment, and Prevention

Edited by
James M. Greenblatt, M.D.
Kelly Brogan, M.D.

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Contents

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Acknowledgments

This book was inspired by all of our patients suffering from inconsistent and ineffective treatments for depression. Our patients' desperate pleas for relief are reminders of our commitment and responsibility as treatment providers. We hope that this textbook will redefine the model for assessment, treatment, and prevention of depression.

We would like to thank all of the contributing authors for donating their time, energy, and passion in producing this textbook. We are fortunate to have collaborated with such a gifted group of dedicated clinicians and researchers in the field of integrative psychiatry to present a new model for future physicians to follow.

We also wish to thank Winnie To for her limitless administrative support and research assistance throughout this endeavor. Her organization, patience, and diligence have been instrumental in the successful completion of this project.

Finally, we would like to express our gratitude to CRC Press for giving us the opportunity and platform to help our colleagues and future physicians understand the utility of integrative approaches toward mental health care. We hope that this textbook will innervate future healthcare leaders to challenge conventional approaches and embrace the evidence-based strategies presented in this text.

James M. Greenblatt, MD and Kelly Brogan, MD, ABIHM

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Foreword

Depression is one of the most serious and costly health problems facing the world today and is the leading cause of death and disability from adolescence through middle age. Approximately 15% of adults will experience severe depressed mood during their lifetimes, and approximately 15% will eventually commit suicide. Depression is the second most costly disorder in the United States, with an estimated annual cost of $80 to $130 billion.[1] Antidepressants are the third most commonly prescribed medications to Americans and the most commonly prescribed medications to Americans between the ages of 18 and 44.[2] In spite of widespread prescribing of antidepressants in developed world regions, the majority of depressed persons in North America and Western Europe do not receive adequate treatment for their symptoms with conventional prescription antidepressants.[3] This is probably the result of poor screening by mental health–care providers, underreporting by patients, and disagreement over uniform criteria for measuring response, remission, relapse, and recurrence of major depressive disorder.[4,5] There is considerable controversy over the efficacy of antidepressants in general as evidenced by the fact that among individuals who are correctly diagnosed and appropriately treated with antidepressants following American Psychiatric Association guidelines, between 40% and 70% do not respond to treatment or respond only partially,[6,7] while roughly half of patients who achieve full remission following treatment for severe depressed mood relapse within 2 years even when continuing on antidepressants.[5,8]

Individuals who do not respond or respond only partially to antidepressants have been labeled "treatment refractory." Because the average difference in drug–placebo response in randomized controlled trials of antidepressants is only 10%, a study on antidepressants in so-called "treatment-refractory" patients, which could potentially yield significant findings, would have to enroll at least 1600 patients.[9] To date neither the pharmaceutical industry nor the National Institutes of Mental Health (NIMH) has funded the large studies needed to evaluate the effectiveness of conventionally used pharmaceutical antidepressants in current widespread use to treat severe depressed mood. One side of the debate contends that the majority of drug industry–sponsored studies on antidepressants have failed to demonstrate significant response differences between antidepressants and placebos,[6,10] while the other side maintains that meta-analyses purporting to show *no benefits* of antidepressants are methodologically flawed or ideologically biased, and that rigorous analysis of findings shows that antidepressants are indeed more effective than placebos.[11] The outcome of the debate over antidepressant efficacy will probably take many more years to resolve and will ultimately rest on expert consensus over complex methodological problems involved in research designs and systematic reviews. The limitations of available conventional treatments of depression suggest that contemporary biomedical models do not adequately explain the causes of major depressive disorder and other mood disorders.

Available antidepressants are limited by adverse effects that reduce adherence, including cognitive impairment, sexual dysfunction, nausea, weight gain, and cardiovascular effects. Other unresolved issues affecting available antidepressants include unfavorable outcomes with long-term use, "paradoxical" depression-inducing effects, antidepressant-induced switching in bipolar disorder, the development of tolerance to beneficial "mood-elevating" effects, reduced efficacy when an antidepressant that was previously effective is tried for recurring depressed mood, and so-called "discontinuation syndromes" when antidepressants are abruptly stopped.

The high cost of many newer antidepressants is a serious obstacle to care for a significant percentage of depressed individuals, especially the elderly and patients on fixed incomes, who cannot afford to use a recommended antidepressant. In response to the high cost of antidepressants, it has been argued that the cost-effectiveness of many older, less expensive antidepressants and expensive recently introduced drugs is roughly equivalent.[12] In addition to general problems related to

the limited efficacy of antidepressants, many depressed patients fail to improve with conventional treatment for several reasons:

- Brief medication visits covered by insurance may preclude adequate assessment and treatment planning.
- Single-drug treatments are often used at inappropriate doses resulting in poor response or high rates of adverse effects, resulting in early discontinuation before a therapeutic regimen has been tried.
- Combining two or more drugs when treating resistant depression frequently leads to a higher incidence of adverse effects, medication nonadherence, and treatment failure.
- When psychiatrists treat patients complaining of depressed mood, psychotherapy is often underutilized or not provided, resulting in diminished treatment response.
- Conventional psychiatric care stresses management of severe depressed mood with relatively less emphasis on maintenance and prevention strategies.

In the context of serious unresolved concerns about the efficacy, safety, tolerability, and affordability of conventional pharmaceutical antidepressants, select alternative treatment modalities of depressed mood are being validated by the findings of placebo-controlled studies, and in some cases systematic reviews of studies. Examples of empirically validated nonpharmaceutical treatments of depressed mood reviewed in this book include select natural products including the herbals St. John's Wort, saffron, and golden root; the vitamin folic acid; a methyl donor called SAMe; the amino acid 5-HTP; omega-3 essential fatty acids; and the pro-hormone DHEA. Select natural products including St. John's Wort, SAMe, 5-HTP, EPA (an omega-3 fatty acid), and acetyl-L-carnitine have been evaluated for their antidepressant efficacy alone or as adjuvants to prescription antidepressants. Accumulating research findings confirm that combining antidepressants with these natural products accelerates the rate of treatment response with few or no safety issues, and improved outcomes. As the authors of this unique volume point out, numerous large, well-designed studies have validated the safety and antidepressant efficacy of SAMe alone and in combination with antidepressants. Folate and vitamin B12 are required cofactors for the synthesis of SAMe, and should be taken together with SAMe for optimal results. It has been established that severely depressed persons found to have low serum folate levels are significantly less likely to respond to antidepressants; therefore, all severely depressed persons should be encouraged to take folate in a form that ensures optimal bioavailability in the brain. Depressed mood also responds to oral doses of the amino acid 5-hydroxy-tryptophan (5-HTP), the immediate precursor of serotonin, taken alone or as an adjuvant to antidepressants. 5-HTP crosses the blood-brain barrier more readily than a related molecule, L-tryptophan, and is converted to serotonin. Similar to a combined regimen of SAMe and an antidepressant, 5-HTP and antidepressants potentiate each other, resulting in a more complete and more rapid response. An important benefit of augmenting an antidepressant with a natural product is the achievement of equivalent response rates at reduced antidepressant dosages resulting in fewer adverse effects and improved medication adherence. Other nonpharmaceutical treatments of mood disorders reviewed in this book that may be safely combined with pharmaceutical antidepressants include exercise, diet, bright light exposure, and mind-body approaches. Other effective nonpharmaceutical treatments of depressed mood include exercise, mind-body therapies such as Yoga, Tai Chi, and Qigong, acupuncture, and bright-light exposure.

Dozens of expert contributing authors of *Integrative Therapies for Depression* have made an unprecedented contribution to the rapidly growing field of integrative mental health care. Through concise reviews of over 3000 published studies discussing cutting-edge research in neurobiology, nutrition, hormonal dysfunction, environmental stresses, and pharmacological and genetic factors in the pathogenesis of mood disorders, this book challenges the current limited biomedical approaches to the assessment of mood disorders. The book summarizes important emerging theories and research findings on the range of nonpharmaceutical therapies used to treat mood disorders,

including vitamins, botanicals and other natural products, exercise, bright light, mind-body practices, and spiritual approaches. Three chapters discussing rational evidence-based approaches to integrative management of mood disorders in pregnant women, adolescents, and the elderly make this textbook unique. This text fills an enormous gap in the conventional model of therapeutics for mood disorders and should be required reading for psychiatrists, psychologists, family therapists, and all clinicians who devote their days to caring for individuals afflicted with melancholia.

James Lake, MD
International Network of Integrative Mental Health

REFERENCES

1. Greden, J.F., Riba, M.B., and McInnis, M.G. 2011. *Treatment Resistant Depression: A Roadmap for Effective Care*. Washington, DC: American Psychiatric Publishing.
2. Pratt, L., Brody, D., Gu, Q. et al. 2011. Antidepressant use in persons aged 12 and over: United States, NCHS Data Brief, No 76. Hyattsville, MD: National Center for Health Statistics.
3. Demyttenaere, K., Bonnewyn, A., Bruffaerts, R. et al. 2008. Clinical factors influencing the prescription of antidepressants and benzodiazepines: Results from the European study of the epidemiology of mental disorders (ESEMeD). *J Affect Disord* 110: 84–93.
4. Simon, G., Fleck, M., Lucas, R. et al. 2004. Prevalence and predictors of depression treatment in an international primary care study. *Am J Psychiatry* 161: 1626–1634.
5. Rush, A.J., Kraemer, H.C., Sackeim, H.A. et al. 2006. Report by the ACNP Task Force on response and remission in major depressive disorder. *Neuropsychopharmacology* 31(9): 1841–1853.
6. Kirsch, I. 2008. Challenging received wisdom: Antidepressants and the placebo effect. *McGill J Med* 11: 219–222.
7. Keitner, G.I. and Mansfield, A.K. 2012. Management of treatment-resistant depression. *Psychiatr Clin North Am* 35(1): 249–265.
8. Schrader, G. 1994. Natural history of chronic depression: Predictors of change in severity over time. *J Affect Disord* 32(3): 219–222.
9. Thase, M. 2006. The failure of evidence-based medicine to guide treatment of antidepressant non-responders. *J Clin Psychiatry* 67(12): 1833–1835.
10. Kirsch, I. 2009. *The Emperor's New Drugs: Exploding the Antidepressant Myth*. London, UK: The Bodley Head.
11. Fountoulakis, K.N. and Möller, H.J. 2012. Antidepressant drugs and the response in the placebo group: The real problem lies in our understanding of the issue. *J Psychopharmacol* 26(5): 744–750.
12. Peveler, R., Kendrick, A., Thompson, C. et al. 2004. Cost-effectiveness of antidepressants in primary care Abstract 9D, Symposium 9, Recent advances in the treatment of psychiatric disorders in primary care: A U.S./U.K. perspective. APA Annual Meeting, New York, NY.

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Editors

James M. Greenblatt, MD, currently serves as Chief Medical Officer and Vice President of Medical Services at Walden Behavioral Care in Waltham, Massachusetts. Walden Behavioral Care is one of the first hospitals in the country to provide a full continuum of care for patients with psychiatric disorders with programs in Massachusetts and Connecticut. Greenblatt is also an assistant clinical professor at Tufts University School of Medicine in Boston, Massachusetts. Prior to joining Walden, Greenblatt founded and served as the Medical Director of Comprehensive Psychiatric Resources, a private psychiatric practice focused on utilizing integrative medicine that has treated patients from all over the world seeking comprehensive psychiatric recommendations.

A pioneer in the field of integrative medicine, Greenblatt has treated patients with mood disorders and complex eating disorders since 1990. After receiving his medical degree and completing his psychiatry residency at George Washington University, Washington, DC, Greenblatt went on to complete a fellowship in child and adolescent psychiatry at Johns Hopkins Medical School, Baltimore, Maryland. He has lectured throughout the United States on the scientific evidence for nutritional interventions in psychiatry and mental illness.

His books, *Answers to Anorexia: A Breakthrough Nutritional Treatment that is Saving Lives* (North Branch, MN: Sunrise River Press, 2010), *The Breakthrough Depression Solution: A Personalized 9-Step Method for Beating the Physical Causes of Your Depression* (North Branch, MN: Sunrise River Press, 2011), and *Answers to Appetite Control: New Hope for Binge Eating and Weight Management* (Boston, MA: James M. Greenblatt, 2014) draw on his many years of clinical experience and expertise in integrative medicine while treating patients with eating and mood disorders. His latest book, *Preserving Memory and Optimal Brain Health*, the first of its kind, discusses the therapeutic use of low-dose lithium. *The Breakthrough Depression Solution* has been translated into Chinese and Japanese and has allowed Greenblatt to train physicians in Asia on complementary therapies for the treatment of mood disorders. Greenblatt's knowledge in the areas of biology, genetics, psychology, and nutrition as they interact in the treatment of mental illness has also led to numerous interviews by the media on television as well as in written articles for consumer audiences.

He continues to provide educational workshops and trainings for physicians across the country. He is committed to educating professionals on the limitations of the current models of treatment for mental illness, and offers practical integrative solutions based on personalized, biochemical testing.

Greenblatt is currently working on his fourth book which will discuss complementary therapies in the treatment of attention deficit-hyperactivity disorder. Greenblatt is also working on a second edition of the *Breakthrough Depression Solution* book for the consumer audience, which will feature exciting new research supporting integrative therapies for the treatment of depression.

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1 Shifting the Paradigm
Redefining the Treatment of Mood Disorders

Emily Deans, MD

CONTENTS

THE ECONOMIC BURDEN OF DEPRESSION

Major depressive disorder is one of the most common mental disorders in the United States and the world. The cardinal criteria involve a period of time in which there is significantly depressed mood or loss of interest or pleasure in addition to problematic changes in functioning in at least four other areas including appetite, sleep, energy, concentration, self-esteem, and suicidal thoughts. In the United States 6.9% of adults (or 16 million people) have experienced at least one episode in the previous 12 months.[1] The World Health Organization estimates 350 million people are affected worldwide, and that in a survey of 17 countries, one in 20 people reported having a depressive episode that past year.[2]

Depressive disorders and other mental illnesses are the leading cause of disability worldwide, accounting for 37% of the healthy years of life lost from noncommunicable diseases, with depression alone accounting for one-third of this burden.[3] The societal costs of loss of mental health and productivity due to depression are also profound, though harder to pin down in a dollar amount. Still, a report from the World Economic Forum estimates the total cost of mental illness globally to be $2.5 trillion in 2010.[4] Depression is sometimes a fatal disease, particularly in combination with the frequently comorbid substance abuse.

Historically, humans have viewed depression and mental illness as caused by anything from possession by supernatural forces to imbalances of humors, with more recent viewpoints postulating that depression symptoms are caused by defects in character, resilience, or poor upbringing. Since the advent of a more biological view of psychiatry, the use of antidepressant medications resulted in a now mostly discredited theory that major depressive disorder was caused by imbalances of neurotransmitters known as the monoamines, including serotonin, norepinephrine, and dopamine.

Our pathologic understanding of the brain, mental illness, and depression in particular has increased exponentially in the past 25 years. New techniques such as functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) scans give us insights into the metabolism of a living brain, while producing neuronal cells from human fibroblasts can help us understand individual genetics and the activity of neurons.

The latest biological explanation of depression, derived from a much better understanding of immunity and the body's stress response system, is the inflammatory model of depression. It is now known that depression is accompanied by chronic, low-grade inflammation, which helps explain its comorbidity with auto-immune disease, obesity, cardiovascular disease, diabetes, and

other inflammatory conditions. In this model, genetic vulnerability, stressful life events, and other modulators including exposure to illnesses in life or in utero, diet, sleep, and exercise all mediate a person's level of resiliency to stress.[5] As perturbations of the system continue to add up, one can develop increasing signs of inflammation along with the symptoms of depression.

LIMITATIONS OF CURRENT TREATMENT MODELS

Despite our advances in knowledge, the recommended treatments for depression have not changed substantially over the previous two decades. The gold standard remains medications (first-line being the selective serotonin reuptake inhibitors or bupropion) in combination with psychotherapy. In general, psychiatric medications despite varying mechanisms will result in a treatment response about 50%–60% of the time, and remission about 30% of the time, with these response numbers plummeting as patients fail the first round of treatments.[6] To compare, placebo response in antidepressant clinical trials hovers around 30%–40%.[7] Some of these numbers are controversial, as it became clear that pharmaceutical companies withheld publication of negative and weak trials of blockbuster antidepressants.[8] Given that antidepressant medications can have serious side effects including agitation, hyponatremia, and severe symptoms upon discontinuation, among others, clinicians must be assured that the treatments they prescribe have more benefit than risk.

Psychotherapy has its own limitations, and the research its own set of problems, including a reluctance to admit that psychotherapy, particularly when poorly chosen or implemented, can also cause side effects.[9] In addition, while the U.S. Food and Drug Administration (FDA) keeps records of unpublished and/or failed trials of antidepressants, there is no such repository of failed psychotherapy trials. Cognitive behavioral therapy has the most data, but in general, comparison of various forms of active therapies (usually psychodynamic, mindfulness-based, or cognitive-behavioral) have shown effectiveness similar to those of antidepressant medications, generally a 50%–60% response rate, with lower total remission of symptoms, and lower rates for those who have already tried treatment before. For more severe cases, however, medication treatments are preferred, and the effectiveness of psychotherapy lessens. With knowledge of industry bias, newer reviews have continued to uphold the slight advantage of combination treatment over either medication or therapy alone.[10,11]

Clinicians and patients are left with a great deal of questions and not a small amount of frustration. Our therapeutic recommendations are based on old data and tainted by publication bias. Even the gold standard fails us nearly half of the time. Each new medication approved for major depressive disorder seems to be a "me-too" pharmaceutical, based on the old monoamine hypothesis. Stigma against mental illness continues to affect research dollars. Despite the massive global burden of depression, the National Insitutes of Health (NIH) put only $415 million into depression research in 2013, compared to $5.3 billion for cancer.[12] With no psychotherapy outperforming any other, and little industry money going to new antidepressant pharmaceuticals given the new (appropriate) scrutiny and perceived saturation of the market, it seems unlikely there will be some sort of fantastic new breakthrough treatment on the horizon.

There is some progress with the approval of some different types of therapeutics for very serious depression, such as transcranial magnetic stimulation and more research into the interesting antidepressant effects of *N*-methyl-D-aspartate (NMDA)-antagonists such as ketamine, but for those of us looking for low-risk, low-cost, outpatient-integrative, not requiring a fancy magnet or an anesthesiologist to work with ketamine infusions, the traditional treatment landscape remains barren. Indeed, the future seems to lie in adequate delivery of treatments and new data helping us with a more personalized and effective approach to treatment.

BRIDGING THE GAP BETWEEN THEORY AND PRACTICE

This book steps in to try to fill in some of those gaps, looking at the evidence base and science behind integrative therapies and personalized medicine for depression and how they might work.

The chapters will explain in far more detail the inflammatory model of depression, and how we can modulate both our immune system and our genetic expression itself with complementary medicine and lifestyle interventions. While some of the risk for major depressive disorder is inherited, or based on factors we cannot control (such as environment in utero), there is much we can still do to decrease the symptoms and improve quality of life beyond the psychotherapy and prescription antidepressant paradigm.

Obviously we cannot alter our genes, but the way in which our genome is expressed (also known as the "epigenome") is another matter entirely. Genes are stored within chromosomes, which are bound on proteins called histones. Various biochemical mechanisms, including methylation, acetylation, and other histone protein modifications will change how, when, and how much a certain protein is made from the genetic code within individual cells and tissues. In general, increasing methylation of histones will "silence" genes, though there are exceptions, and the complete epigenetic modulation of promotor regions, genes, and gene expression is exceedingly complex and poorly understood.

Medications can alter epigenetic expression. Monoamine oxidase inhibitors are potent inhibitors of histone demethylase LSD1, and valproate, used as a mood stabilizer, is an inhibitor of histone deacetylases. Monozygotic twins have a large accumulation of epigenetic differences as they age, giving evidence that different environmental experiences alter the expression of genes and explain how psychotherapeutic, lifestyle, and medication interventions can impact the biologic expression of depression symptoms.[13]

With our growing knowledge of inflammation, epigenetics, and personalized medicine, it is possible to help our patients in a scientific and evidence-based manner despite the limitations of the standard treatment paradigm. This textbook is an exploration of clinically relevant lifestyle and integrative medicine techniques that may be used in a meaningful way by clinicians seeking to better serve their patient with depressive disorders.

REFERENCES

1. National Institute of Mental Health, *12-Month Prevalence of Major Depressive Episode Among U.S. Adults 2012*. http://www.nimh.nih.gov/health/statistics/prevalence/major-depression-among-adults.shtml. Accessed December 28, 2014.
2. Marcus, M., Yasamy, T., Ommeren, M.V. et al. 2012. *Depression: A Global Public Health Concern*. World Health Organization, Department of Mental Health and Substance Abuse.
3. World Health Organization. 2008. *The Global Burden of Disease*, 2004 Update.
4. Bloom, D.E., Cafiero, E.T., Jané-Llopis, E. et al. 2011. *The Global Economic Burden of Noncommunicable Diseases*. Geneva: World Economic Forum.
5. Berk, M., Williams, L.J., Jacka, F.N. et al. 2013. So depression is an inflammatory disease, but where does the inflammation come from? *BMC Med* 11: 200.
6. Trivedi, M.H., Rush, J.A., Wisniewski, S.R. et al. 2006. Evaluation of outcomes with citalopram for depression using measurement-based care in STAR*D: Implications for clinical practice. *Am J Psychiatry* 163(1): 28–40.
7. Sonawella, S.B. and Rosenbauam, J.F. 2002. Placebo response in depression. *Dialogues Clin Neurosci* 4(1): 105–113.
8. Turner, E.H., Matthews, A.M., Lindardatos, E. et al. 2008. Selective publication of antidepressant trials and its influence on apparent efficacy. *N Engl J Med* 358: 252–260.
9. Barlow, D.H. 2010. Negative effects from psychological treatments. *Am Psychol* 65: 13–19.
10. DeRuebis, R.J., Siegle, G.J., and Hollon, S.D. 2008. Cognitive therapy vs. medications for depression: Treatment outcomes and neural mechanisms. *Nat Rev Neurosci* 9(10): 788–796.
11. Cuijpers, P., Turner, E.H., Mohr, D.C. et al. 2014. Comparison of psychotherapies for adult depression to pill placebo control groups: A meta-analysis. *Psychol Med* 44(4): 685–695.
12. Ledford, H. 2014. If depression were cancer. *Nature* 515: 182–184.
13. Sun, H., Kennedy, P.J., and Nestler, E.J. 2013. Epigenetics of the depressed brain: Role of histone acetylation and methylation. *Neuropsychopharmacol Rev* 38(1): 124–137.

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2 The Role of Inflammation in Depression

Antolin C. Trinidad, MD, PhD

CONTENTS

INTRODUCTION

One of the most enduring core concepts in understanding clinical depression or major depressive disorder (MDD) and how it differs from other types of depression are the so-called concepts of vegetative signs and symptoms as comprising the essential features of MDD: lethargy, sleep disturbances, concentration problems, and somatic preoccupations that are not associated with observable or detectable physical abnormalities. Aches, pains, and "flu-like" symptoms are common complaints among depressed people, as is the admission: "I just do not feel that I am myself anymore." These same signs and symptoms are reproducible in situations where patients receive interferon therapy such as when they are in treatment for hepatitis C and certain forms of cancer.[1] Other symptoms in interferon therapies for these disorders can rapidly develop, such as pain, sleep disorders, and appetite problems, along with suicidal thinking. These adverse effects are considered major side effects of these treatments. Psychiatric involvement is often required or highly recommended. Interferon is a glycoprotein, in a class of compounds termed *cytokines*. It has functions in the human immune system that range from antiviral to the activation of natural killer cells and macrophages.[2] Disorders characterized by heightened activity of the immune system such as systemic lupus erythematosus, other connective tissue disorders, and coronary artery disease are associated with a high prevalence of depression.

In part because of the ability of interferon to induce MDD-like manifestations, investigators then questioned whether MDD itself is associated with, if not caused by, inflammation. Several layers of evidence now strongly implicate inflammatory mechanisms in depression. Inflammatory cytokines are elevated in the peripheral blood and cerebrospinal fluid (CSF) of people with major depression who are physically healthy.[3] Cytokines interact with many central nervous system (CNS) functions including neurotransmitter metabolism, neuroendocrine regulation, and neural plasticity. Cytokines affect the synthesis and usage of neurotransmitters that modulate mood such as the monoamines serotonin, norepinephrine, and dopamine. Some effects of increased cytokines associated with major depressive disorders (MDDs) include

- Influences the release and reuptake of monoamines
- Influences the hypothalamic-pituitary-adrenal (HPA) axis and the secretion of corticotropin-releasing hormones (CRHs)
- Decreases neurogenesis and increases apoptosis
- Impairs neuronal branching
- Decreases neuronal plasticity
- Diverges kynurenine pathway of tryptophan metabolism into a neurotoxic path

When administered to humans, cytokines stimulate the release of CRHs that then stimulate the release of adrenocorticotropic hormone (ACTH); both CRH and ACTH are known to be elevated in MDD. Although a certain level of cytokines may have trophic effects to neurons and may promote neurogenesis, an excessive level or activity of cytokines can do the opposite—it can increase apoptosis and reduce neurogenesis in some areas such as the hippocampus.[4]

HYPERAROUSAL OF THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS

In patients who have a clear-cut physical reason for inflammatory processes like autoimmune disease or even exogenous cytokine/interferon administration for therapeutic purposes, the cluster of signs and symptoms of depression may be explainable through these physiological processes. However, in patients with only MDD as the disorder without an identifiable physical abnormality, mere stress can trigger the cascade of inflammatory processes.[5]

Both the sympathetic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis mediate these responses through the pulsatile release of catecholamines that can activate inflammatory signaling pathways. Although cortisol is an anti-inflammatory hormone, chronic stress can make the organism cortisol resistant. In a chronically stressed organism, high levels of cortisol are an index of both the chronic activation of the HPA axis and that organism's relative resistance to cortisol effects. Translating these findings to clinically relevant observations, hyperarousal, irritability, anxiety, and sleep disturbances are frequently seen in depression. These symptoms can be explained by a hyperarousal of the HPA axis. Depression also comes often with cognitive decline, which can be the result of cytokine-induced CNS apoptosis. Clinical and preclinical studies show that stress and depression are associated with neuronal loss in the hippocampus and limbic structures.[6,7]

While hyperarousal and associated symptoms such as anxiety and decreased sleep may promote behaviors associated with a fight-or-flight response potentially crucial for survival, they come with a price. The evidence suggests that certain structures of the central nervous system may be "worn down" through time, and that depression is a syndrome that may be a downstream effect of this process that starts much earlier. Inflammation may be the mechanism by which these processes work their way in the organism. In depression, the chronic inflammatory mechanisms themselves can induce structural changes in the brain such as hippocampal apoptosis. In turn, such structural changes can perpetuate organismal inability to cope with the stress, because the organism is less able to solve problems effectively (an example of which are the cognitive deficits that happen in major depression), thus perpetuating the cycle. This process is schematized in Figure 2.1.

However, this mechanism underlies situations wherein chronic stress happens such as in inescapable, threatening situations occurring over a period of time. Depression happens even in situations where such a dynamic is not present. In those cases, the likely mechanism is an inherently dysfunctioning inflammatory process, or an exogenously induced depression such as the administration of interferon-α in patients undergoing hepatitis C treatment.

Depression is of course heterogeneous in causation and too complex to be understood solely on the basis of one mechanism. Sex is a factor that affects the depression and inflammation relationship. It has been pointed out that the association of inflammatory markers with depression is more robust among men than it is among women.[8,9] The association of inflammation with metabolic syndrome

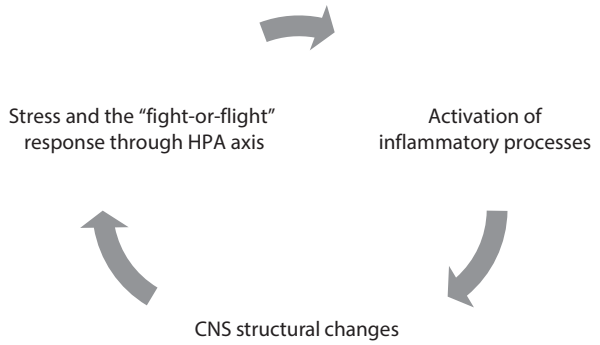

FIGURE 2.1 How stress produces CNS structural changes.

and the amount of visceral fat has been speculated as one possible mechanism—it is known that visceral fat cells release cytokines and that metabolic syndrome has been associated with increased inflammatory markers. Indeed, adipose tissues, long thought to be relatively passive storage sites of fat, are now known to function as glands, secreting various hormones and metabolically regulating substances. Because men tend to have higher quantities of visceral fat, this may explain the association with inflammation and depression as attaining a higher rate in men than in women. Other pro-inflammatory lifestyle factors such as cigarette smoking and alcohol use may be important factors as well in scrutinizing the intersection between gender, depression, and inflammation.

ACUTE AND CHRONIC INFLAMMATION

It is useful to distinguish between acute and chronic inflammation and the corresponding cascades of inflammatory changes in each category. One model of acute inflammation is experimental sepsis. In the latter state, the cytokine cascade includes tumor necrosis factor α (TNF-α), interleukin 1β (IL-1β), IL-6, IL-1ra, soluble TNF-R, IL-10, and C-reactive protein. TNF-α and IL-1 stimulate the production of IL-6, which has both pro-inflammatory and anti-inflammatory functions. In chronic, low-grade inflammation, the origin of TNF is thought to be adipose tissue. The levels of these inflammatory markers may increase several-fold, then decrease as the infection subsides. Chronic, low-grade inflammation is a state hypothesized to occur when there is a two- to threefold increase in these levels (lower amplitude than in sepsis models) over a period of time. Chronic, low-grade inflammation occurs with chronic medical disorders like diabetes, obesity, cardiac disease, and aging. In a study of elderly, depressed subjects, it has been hypothesized that the chronic inflammatory profile co-occurs with the activity of two enzymatic pathways, the indoleamine-2,3-dioxygenase (IDO) and the guanosine-triphosphate-cyclohydrolase-1 (GTP-CH1) pathways, both of which are involved in the biosynthesis of monoamines. This finding is related to increased tryptophan catabolism in turn associated with the depressive symptoms of lassitude, reduced motivation, anorexia, and pessimism. In contrast, variations in markers of GTP-CH1 activity correlated more with neurovegetative symptoms, including sleep disturbance, digestive symptoms, fatigue, sickness, and motor symptoms.[10]

Because there are high levels of circulating cytokines in obesity, it can be considered a state of chronic inflammation with or without depression. But what exactly is the relationship between obesity, depression, and inflammation? In an attempt to answer this question, a structural equation model, which makes it possible to examine the validity of competing models specifying different patterns of relations among variables, examined the relations among depression, adiposity, leptin levels, and inflammation. Using this method, there is support for a model that points to depressive symptoms promoting weight accumulation, which in turn activates an inflammatory response—in other words, the depression seems to come first, then the weight gain.[11] Indeed, depression has been

associated with significant reduction in physical activity among those who are depressed.[12,13] There is also evidence that the relationship may be bidirectional: depression increases the risk for obesity, but obesity also increases the risk for depression.[14] But, other studies do not support such linear relationships—some studies question whether there is a relationship between obesity and depression at all. Despite these questions, there is a strong association in the literature between significant or extreme obesity and depression. There is also family correlation. Multivariate logistic regression analyses in a sample of 482 nuclear families segregating extreme obesity and normal weight indicated that body mass index (BMI), race, marital status, chronic medical conditions, and family history were the predicators of depression for both the genders. Thus, those with family histories of depression seem to have higher risks of obesity as well.[15] The summative idea seems to be that whatever the basic pathophysiologic mechanism is, there are intimate correlations between lowered physical activity, obesity, depression, and measures of chronic inflammation.

RESTORING HOMEOSTATIC MECHANISMS: THE ROLES OF ANTIDEPRESSANTS, EXERCISE, AND MEDITATION

The monoamine theory of depression has been the prevailing and dominant theory that explained a possible biochemical basis for major depression during the latter parts of the twentieth century. The theory posits that there lies a disorder in monoaminergic neurotransmission in the pathophysiology of major depression. The monoamine theory is at best simplistic and underplays what is involved in complex central nervous system processes that modulate stress effects. It also inadequately addresses the attendant restorative mechanisms to attain homeostasis. Inflammatory mechanisms are a sign that there exists a lack of or failing homeostasis underlying depression. To restore homeostasis thus becomes of paramount importance when considering a road to recovery. Three factors that potentially complement each other in restoring homeostatic mechanisms to counteract the adverse effects of inflammation are antidepressants, physical exercise, and meditation.

In the monoamine theory of depression, the role of antidepressants in MDD treatment has originally been conceptualized as primarily occurring through their actions on synaptic transmissions in serotonergic, noradrenergic, and dopaminergic systems in the central nervous system. Though intracellular mechanisms are also thought to be important factors in the mechanisms of antidepressant action—for example, in the case of lithium as an adjunctive/adjuvant element in certain types of depression whose actions are primarily in the cytoplasm—the specific site that has gathered the most interest is transmission at the synaptic junction. But with the recognition of inflammation as a potential factor in depression, antidepressant mechanisms beyond the synapse have been re-evaluated. In an attempt to directly attenuate inflammation in some selective serotonin reuptake inhibitor (SSRI) treatment nonresponders, the addition of celecoxib, an anti-inflammatory drug, produced greater antidepressant response, although further replication studies of this paradigm with a larger number of subjects remain lacking.[16] Again, beyond their specific actions at the synapse, preclinical and clinical studies implicate SSRI effects on various steps in the inflammatory response including the reduction of levels of TNF-α, an acute-phase cytokine produced by macrophages, and various interleukins. In one study, a group of subjects with major depression has a higher level of pretreatment TNF-α compared to controls; in post-treatment with sertraline, there was a lowering of these levels compared to those who were not treated.[17] Moreover, administration of the TNF-α antagonist etanercept to patients with moderate to severe psoriasis and ankylosing spondylitis resulted in improved symptoms of depression measured by the Beck Depression Inventory. This improvement was independent of the effect of etanercept on joint pain.[18] Although there are studies that conflict with the finding of SSRIs lowering interleukin levels, meta-analysis of the role of antidepressants generally suggests a positive correlation. The SSRIs as an antidepressant class have been shown to be particularly able to lower levels of IL-1β and IL-6. There is clinical correlation in this relationship in that the lowered levels of these interleukins also significantly lower the severity of depression in the studies that were analyzed.[19]

NEUROINFLAMMATION

Another area where the intersection of stress, depression, immunity, and antidepressant action lies is on hippocampal neurogenesis. First, neurogenesis, or the formation of new neurons, is blocked by inflammation alone. Clinical states involving CNS inflammation reduce neurogenesis, and in some cases, this effect can be diminished by anti-inflammatory drugs alone. This finding was reported in rats that underwent a bacterial lipopolysaccharide injection to induce inflammation. Neuroinflammation was blocked by indomethacin.[20] It is known that apoptosis in the hippocampus accelerates during chronic stress. Such chronicity contributes to atrophy of hippocampal structures, mediated by the HPA axis which in turn is influenced by inflammatory mechanisms. In major depression, this factor is hypothesized to underlie the cognitive decline that can accompany it as a symptom. It seems that in contrast to the effects of stress on the hippocampus, the chronic administration of antidepressants (both serotonergic and noradrenergic) increases neurogenesis or the development of new neurons. A crucial factor is chronic administration—this agrees with the well-known observation that it takes time for antidepressants to effectuate clinically observable symptomatic benefits. The subgranular zone (SGZ) of the hippocampus is one of two major neurogenic zones. Neural progenitors in the adult brain give rise to new neurons; these progenitors are particularly plentiful in the SGZ. Atypical antipsychotics, often used for antidepressant purposes, promote proliferation of neural progenitors in the SGZ and the survival of these progenitors into mature neurons. Older antipsychotics do not have these effects.[21]

PHYSICAL EXERCISE

It has been shown by several studies that physical exercise alone may have robust antidepressant properties. Exercise also promotes neurogenesis. The practical benefits of enhanced neurogenesis are most visible in aging subjects where physical exercise enhances learning and memory and executive function, and counteracts age-related brain atrophy in certain areas.[22] In the hippocampus where enhanced neurogenesis contributes to positive cognitive change, there are at least two growth factors mediating synaptic plasticity: brain-derived neurotrophic factor (BDNF) and insulin-like growth factor-1 (IGF-1). In animal studies, exercise increases BDNF in several brain regions. IGF-1 gene expression in turn is increased in hippocampal neurons as a result of exercise. There are also some suggestions that both BDNF and IGF-1 mediate the antidepressant and anxiolytic effects of exercise. While CNS IGF-1 is a growth factor, it also affects peripheral insulin functioning, in that exercise increases peripheral IGF-1, which then leads to improved insulin sensitivity. Pro-inflammatory cytokines from whatever etiology impair IGF-1 functioning; regular exercise reduces circulating pro-inflammatory cytokines, thereby restoring impaired IGF-1 functioning.[23]

There is also a relationship between exercise and the body's reaction to infection. A novel experiment to investigate this relationship is the immune activation induced by experimental infection, the infusion of low-dose *Escherichia coli*. There is a two- to threefold increase in TNF-α. If these same subjects were then subjected to 3 hours of ergonomic cycling, this burst is blunted, suggesting that exercise suppresses endotoxin-induced rise of TNF-α. The cytokine IL-6 has a unique function in that it induces anti-inflammatory cytokines such as IL-1-ra and IL-10. IL-6 also suppresses TNF-α production in in vitro studies. During rapid bursts of exercise, there is a rise of IL-6. Thus, even short bursts of exercise have shown to cause changes that can be deemed anti-inflammatory.[24]

It is, however, the customary recommendation for exercise to be regular and sustained and not just activities to be done in random fits and starts. How does long-term, regular exercise change the immunologic picture? Studies on elite swimmers in training showed lowered immunoglobulin measures.[25] These finding have been confirmed by earlier studies on athletes and intense exercise; there seems to be a lowering of lymphocytes, suppressed natural immunity, and high levels of pro-inflammatory cytokines likely associated with muscle damage. Such a dynamic brings to question the ability of intense exercise to allow subjects to resist infection. But it is likely that in sustained

moderate exercise, there is greater recruitment of the HPA axis to respond and be a greater factor in the modulation of the totality of immunity. Chronic exposure to increased cytokines blunts the cortisol response. In addition to it, sustained, moderate exercise mediates the anti-inflammatory dynamic by the reduction of visceral fat mass and the reduction of circulating adipokines.[26] At this point in the discussion, the interlocking factors of physical exercise, adiposity/obesity, immune functions, and depression can be adequately appreciated: an increase in physical activity over a period of time reduces adiposity which then decreases the risk of depression, a state that is reflected in the changing homeostasis of immune measures.

MEDITATION

In terms of mental homeostasis, or keeping the mind at a steady, nonstressed condition despite the unpredictable vicissitudes of daily life, the ancient practice of meditation, traditionally associated with Asian culture and philosophy, has been known to contribute to mental well-being among its practitioners. Its role in contemporary treatments of mental illness, especially depression and anxiety, effloresced not only because of migration and a more rapid transmission of cultural practices, but also because Western-derived medical modes of healing such as pharmacological interventions have borne imperfect results in many instances; they have also induced side effects that can be intolerable to some. Many of those who suffer from mental illnesses turn to methods or interventions that deviate from the strictly biomedical models. Meditation for depression has been shown effective either as an adjunct or by itself. In discussing this intervention, it is important to start from a more basic point, which is defining what meditation is. Such a definition is crucial to discussing its effects on inflammation. Meditation is often confused with the generic phrase "relaxation exercises." However, meditation is most associated with therapeutic benefits that stem from physiologic mechanisms involving the nexus of the cortex, the HPA axis, and the immune system. For these purposes, the definition offered by Jonathan Nash and Andrew Newberg is especially cogent. They define meditation as contemplative practices that relate to "either a family of mental training techniques (the 'method definition'), or in relation to the particular altered states of consciousness that arise from the implementation of the technique (the 'state definition')".[27] They propose the inclusion of both method and state as parts of the same dynamic process that unfolds over time. The "method" thus attains the "state," which is an enhanced mental state (EMS) akin to an alteration in consciousness associated with feelings of enhanced well-being, knowledge, bliss, focus, and detachment. Such a definition relates meditation as methods and states that address levels of consciousness, alertness, and awareness. These levels in turn can be thought of as variations of cortical function. As such, the neurobiological correlates of meditation have been amply documented by functional magnetic resonance imaging (MRI) and electroencephalogram (EEG). Cortical and subcortical areas of the brain implicated in meditative states are summarized by Tobias Esch.[28]

One of the pathways by which the contemplative state attained through meditation or the EMS is by downregulation of the HPA axis. Evidence for this can be gleaned from lowered cortisol in subjects who undergo meditation.[29,30] Because meditation is a heightened function of focus and attention at least in one of its stages, this process activates the prefrontal area, particularly the right side, and the cingulate cortex. It also activates the limbic system which has rich interconnections with the hypothalamus, which then connects with the parasympathetic system to affect heart rate and respiration rate and to facilitate a sense of quiescence achieved during meditation. The locus ceruleus (LC) connects with the hypothalamus as well; activation of the LC increases the release of CRH.[31] Inhibition of the release of CRH then effectuates a corresponding change in the immunologic environment. A pool of studies show benefits of various methods of meditation on depression, anxiety, and other forms of psychological or psychiatric states.[32]

Measures of inflammatory function and the immune system have recently been included in studies of meditation as an intervention for stress, distress, and depression in an effort to study these effects at the cellular level. In a subject pool of dementia caregivers, a brief daily yogic meditation

intervention reversed the pattern of increased NF (nuclear factor)-κB-related transcription of pro-inflammatory cytokines and decreased IRF1 (interferon response factor 1)-related transcription of innate antiviral response genes previously observed in healthy individuals.[33] Another study also focused on a sample of dementia caregivers; the study group, whose average age is 60 years old, underwent yogic meditation while the controls underwent relaxation training. Results showed not only significant differences in the Hamilton Depression Rating Scale (Ham-D) scores but also differences in telomerase activity, with the study group showing significantly improved telomerase activity. Telomere length has been used as a "psychobiomarker" linking chronic psychological stress and diseases in aging. A telomere is a region of repetitive DNA sequences at the end of a chromosome, which protects the end of the chromosome from deterioration. Shortened telomere length and reduced telomerase are risk factors for shortened mortality and also predict several other health and stress issues, in turn, indicative of psychological stress.[34] These findings speak to other findings in the literature suggesting that the association between inflammatory markers and depression attain a stronger relationship in aging populations, and that interventions to diminish depression and/or inflammation connote compelling results in this population.

CONCLUSION

The mental health professions have been used to conceptualizing MDD as a categorical clinical state with a set of signs and symptoms uniquely characterizing it. Whatever the physiological correlates were associated with MDD that have been observed in previous investigations, there was an implicit idea that they were epiphenomena, meaning they occur because of an independent disorder operating to produce these physiological correlates. The monoamine hypothesis was thought to be a crucial mechanism underlying MDD—it is now suspect whether it is even correct as a sole explanation. Since the discovery that measures of inflammatory states are altered in depression along with the discovery that signs and symptoms of MDD can be reproduced by the infusion of cytokines, the reverse idea has gained traction. It is now entirely possible that what we call MDD is the epiphenomenon—a constellation of signs and symptoms of an underlying bigger disorder whose clinical manifestations subsume obesity, cardiac diseases, and other chronic inflammatory conditions. The physiological nexus that entangles these conditions is a chronic dysfunction of the HPA axis and its attendant stoking of inflammatory function aberrancies. What is clear is that in terms of therapies, antidepressant medications, especially SSRIs, are a complementary (see Figure 2.2), not contradictory, intervention to attenuate the chronic inflammatory malfunction along with physical exercise and meditation.

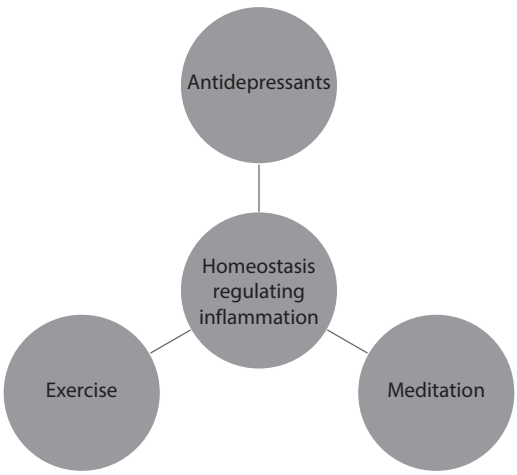

FIGURE 2.2 Complementary functions of antidepressants, exercise, and meditation.

REFERENCES

1. Koh, C., Heller, T., Haynes-Williams, V. et al. 2013. Long-term outcome of chronic hepatitis C after sustained virological response to interferon-based therapy. *Aliment Pharmacol Ther* 37(9): 887–894.
2. Gonzales-Navajas, J., Lee, J., David, M. et al. 2012. Immunomodulatory functions of type 1 interferons. *Nature Rev Immunol* 12: 125–135.
3. Raison, C.L. and Miller, A.H. 2011. Is depression an inflammatory disorder? *Curr Psychiatry Rep* 13: 467–475.
4. Kubera, M., Obuchowicz, E., Goehler, L. et al. 2010. In animal models, psychosocial stress-induced (neuro) inflammation, apoptosis and reduced neurogenesis are associated to the onset of depression. *Prog Neuropsychopharmacol Biol Psychiatry* 35(3): 744–759.
5. Zunszain, P.A., Anacker, C., Cattaneo, A. et al. 2011. Glucocorticoids, cytokines, and brain abnormalities in depression. *Prog Neuropsychopharmacol Biol Psychiatry* 35(3): 722–729.
6. Duman, R.S. 2004. Depression: A case of neuronal life and death? *Biol Psychiatry* 56(3): 140–145.
7. Lucassen, P., Meerlo, P., Naylor, A.S. et al. 2010. Regulation of adult neurogenesis by stress, sleep disruption, exercise and inflammation: Implications for depression and antidepressant action. *Eur Neuropsychopharmacol* 20: 1–17.
8. Vogelzangs, N., Duivis, H.E., Beekman, A.T.F. et al. 2012. Association of depressive disorders, depression characteristics and antidepressant medication with inflammation. *Transl Psychiatry* 2: 1–9.
9. Vogelzangs, N., Kritchevsky, S.B., Beekman, A.T.F. et al. 2008. Depressive symptoms and change in abdominal obesity in older persons. *Arch Gen Psychiatry* 65: 1386–1393.
10. Capuron, L., Schroecksnadel, S., Feart, C. et al. 2011. Chronic low-grade inflammation in elderly persons is associated with altered tryptophan and tyrosine metabolism: Role in neuropsychiatric symptoms. *Biol Psychiatry* 70(2): 175–182.
11. Miller, G.E., Freedland, K.E., Carney, R.M. et al. 2003. Pathways linking depression, adiposity, and inflammatory markers in healthy young adults. *Brain Behav Immun* 17(4): 276–285.
12. Simon, G.S., Ludman, E.J., and Linde, J.A. 2008. Association between obesity and depression in middle-aged women. *Gen Hosp Psychiatry* 30(1): 32–39.
13. Dixon, J.B., Dixon, M.E., and O'Brien, P.E. 2003. Depression in association with severe obesity changes with weight loss. *Arch Intern Med* 163(17): 2058–2065.
14. Luppino, F.S., de Wit, L.M., Bouvy, P. et al. 2010. Overweight, obesity, and depression a systematic review and meta-analysis of longitudinal studies. *Arch Gen Psychiatry* 67(3): 220–229.
15. Dong, C., Sanchez, L.E., and Price, R.A. 2004. Relationship of obesity to depression: A family-based study. *Int J Obesity* 28: 790–795.
16. Abbasia, S., Hosseinia, F., Modabberniac, A. et al. 2012. Effect of celecoxib add-on treatment on symptoms and serum IL-6 concentrations in patients with major depressive disorder: Randomized double-blind placebo-controlled study. *J Affect Disord* 141(2–3): 308–314.
17. Sutcigil, L., Oktenli, C., Musabak, U. et al. 2007. Pro- and anti-inflammatory cytokine balance in major depression: Effect of sertraline therapy. *Clin Dev Immunol* 2007: 76396.
18. Arisoy, O., Beş, C., Çifci, C. et al. 2011. FC09-05—The effect of TNFα blockers on psychometric measures in treatment resistant ankylosing spondylitis patients: A pilot study. *Eur Psychiatry* 26(1): 1862.
19. Hannestad, J., DellaGioia, N., and Bloch, M. 2011. The effect of antidepressant medication treatment on serum levels of inflammatory cytokines: A meta-analysis. *Neuropsychopharmacology* 36: 2452–2459.
20. Monje, M.L., Toda, H., and Palme, T.D. 2003. Inflammatory blockade restores adult hippocampal neurogenesis. *Science* 302: 1760–1764.
21. Manev, H., Uz, T., Smalheiser, N.R. et al. 2001. Antidepressants alter cell proliferation in the adult brain in vivo and in neural cultures in vitro. *Eur J Pharmacol* 411(1–2): 67–70.
22. Cotman, C.W., Berchtold, N.C., and Christie, L. 2007. Exercise builds brain health: Key roles of growth factor cascades and inflammation. *Trends Neurosci* 30(9): 464–475.
23. Duman, R.S. 2005. Neurotrophic factors and regulation of mood: Role of exercise, diet and metabolism. *Neurobiol Aging* 26(1): 88–93.
24. Petersen, A.M.W. and Pedersen, B.K. 2005. The anti-inflammatory effect of exercise. *J Appl Physiol* 98: 1154–1162.
25. Gleeson, M., McDonald, W.A., Pyne, D.B. et al. 2000. Immune status and respiratory illness for elite swimmers during a 12-week training cycle. *Int J Sports Med* 21(4): 302–307.
26. Gleeson, M., Bishop, N.C., Stensel, D.J. et al. 2011. The anti-inflammatory effects of exercise: Mechanisms and implications for the prevention and treatment of disease. *Nature Rev Immunol* 11: 607–615.

27. Nash, J.D. and Newberg, A. 2013. Toward a unifying taxonomy and definition for meditation. *Frontiers Psychol* 4: 1–17.
28. Esch, T. 2014. The neurobiology of meditation and mindfulness. *Studies Neurosci Consciousness Spirituality* 2: 153–173.
29. Walton, K.G., Fields, J.Z., and Levitsky, D.K. 2004. Lowering cortisol and CVD risk in postmenopausal women: A pilot study using the transcendental meditation program. *Ann NY Acad Sci* 1032: 211–215.
30. Matouseka, R.H., Dobkina, P.L., and Pruessnerb, J. 2010. Cortisol as a marker for improvement in mindfulness-based stress reduction. *Complement Ther Clin Pract* 16(1): 13–19.
31. Newberg, A. and Iversen, J. 2003. The neural basis of the complex mental task of meditation: Neurotransmitter and neurochemical considerations. *Med Hypotheses* 61(2): 282–291.
32. Hofmann, S.G., Sawyer, A.T., Witt, A.A. et al. 2010. The effect of mindfulness-based therapy on anxiety and depression: A meta-analytic review. *J Consult Clin Psychol* 78(2): 169–183.
33. Black, D.S., Cole, S.W., and Irwin, M.R. 2013. Yogic meditation reverses NF-κB and IRF-related transcriptome dynamics in leukocytes of family dementia caregivers in a randomized controlled trial. *Psychoneuroendocrinology* 38(3): 348–355.
34. Lavretsky, H., Epel, E.S., Siddarth, P. et al. 2013. A pilot study of yogic meditation for family dementia caregivers with depressive symptoms: Effects on mental health, cognition, and telomerase activity. *Int J Geriatr Psychiatry* 28(1): 57–65.

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3 The Gut-Brain Axis
The Role of the Gut in Brain Health

Court Vreeland, MS, DC, DACNB and
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CONTENTS

INTRODUCTION

Current psychiatric care has failed many patients with mood disturbances, and perhaps it's because the paradigm is incorrect. In many ways, modern psychiatry has served as a repository for the diagnostic and therapeutic limitations of conventional medicine. When a patient's symptoms of malaise, brain fog, lethargy, inattention, insomnia, agitation, and flat mood slip through the cracks of the discrete territories of specialty medicine, patients are referred for psychiatric prescription treatment. When they are treated with nonsteroidal anti-inflammatory drugs (NSAIDs), statins, acid blockers, antibiotics, and birth control pills, the mechanistic insults of these medications are poorly appreciated by prescribers, side effects are dismissed, and patients are, again, referred for psychiatric care. What happens when psychiatric care itself is predicated on medication treatment with placebo-driven short-term effects,[1–3] and worse functional outcomes in the long term?[4–6]

Escalating incidence of mood disorders may be attributable to multiple sources. Some of these include socioeconomic changes, urbanicity, dietary changes (Western-style diet), sedentary behavior, excessive screen-based information consumption, lack of adequate sunlight, reduced offline social support, and an overall disconnect from nature.[7]

The impact of these lifestyle changes may be found in transgenerational shifts to the gut microbiome—the ecology of microbes that inhabit our gastrointestinal (GI) system. Research, both recent and from the turn of the twentieth century, indicates that proper GI function might also play a role in mood disorder. Through several factors like microbial balance, gut barrier integrity, immune stimulation, and altered systemic inflammatory load, the health of the gut is increasingly being seen as vital to mental health.

GUT-BRAIN AXIS COMPONENTS

The gut-brain axis is complex and involves the following systems: central nervous system (CNS), neuroendocrine system, neuroimmune system, sympathetic and parasympathetic nervous systems, enteric nervous system, and intestinal microbiota.[8] Alterations of the microbiota with the stresses of everyday life and the Western lifestyle are thought to play a key role. The microbiota is best viewed as a type of inner organ. It is widely known that disruptions in the function of other organs may cause changes in CNS function. The same applies to disruptions of the microbiota. It has long been held that human fetuses are sterile at birth. This medical dogma is changing as of late with some studies on neonatal meconium showing the presence of microbial DNA.[9] Even the placenta has been found to harbor its own unique microbial fingerprint.[10] These findings suggest an integral role for microbiota from fetal to infant development that set the stage for adult health. If that is not convincing of the relevance of microbial contribution, then the sheer size of the numbers of organisms present should be.

By the age of one, the microbial profile looks similar to that of an adult, although it maintains its own individuality with respect to others.[11] Total adult load is estimated at 1800 genera, 40,000 species, 1–2 kg in weight, 100 trillion in number,[12] and possesses 100 times the genes found in the human genome.[13] Established functions of microbiota on human health are known and include regulation of the mucosal immune system, regulation of GI motility, epithelial barrier regulation, digestive and metabolic support including neurotransmitter production, and the prevention of colonization by pathogens.[8] It is perhaps through disruptions of these functions, disruptions of some yet undiscovered connection, or more likely, disruptions of a combination of these factors that changes in CNS functioning may be seen.

HISTORICAL PERSPECTIVES

It was not all that long ago that most of medicine viewed GI hyperpermeability as pseudoscience. Even less credence was given to how this permeability might affect the rest of the body or the brain. However, as far back as the early twentieth century it was suggested that microbial balance, and its subsequent purposeful manipulation, could positively affect mood. It was not uncommon to see the terms *autointoxication*, *intestinal stasis*, or *intestinal toxemia* in medical journals. Respected medical journals used the terms interchangeably, although *autointoxication* held great favor among authors. Scientists connected these phenomena to many things; however, most common among them were fatigue and mood change. While terms like *autointoxication*, *intestinal stasis*, and *intestinal toxemia* fell out of favor, and their mechanisms proved to be inaccurate, the idea of a connection between GI problems and mood never completely disappeared. Although the turn of the twentieth century was rife with what proved to be the half-truths of autointoxication, some scientists were laying the groundwork for the field of study we know today.

As early as 1904 it was observed that institutionalized adults (for mania or melancholia) had an increased blood reaction to intestinally derived *Escherichia coli*, suggesting the presence of preexisting immune priming to intestinal bacteria. In 1906, a physician from New York named Fenton Turck reported that psychological stress in laboratory animals was capable of altering gut permeability. Similarly, he showed that dietary factors could also affect permeability. In an experiment in which he fried bread in cottonseed oil for 30 minutes (likely producing trans fatty acids) and fed it to laboratory animals, he found increases in intestinal permeability as compared to control animals.

Other authors focused on the role of low-grade immune response in the genesis of melancholia. The idea of immune response and melancholia was eventually linked in 1920. Even the idea of endotoxins (an accepted concept today) has its origins 100 years ago. Turck believed these endotoxins were produced by intestinal bacteria and could get out of the GI tract, inducing systemic illness. Most of the research of the day regarding endotoxins focused on their roles in GI disorders like food poisoning. In 1913, microbiologist Arcangelo Distaso stated that nervous disorders that

also included constipation and/or diarrhea as components seemed to be connected to the liberation of endotoxins from gram-negative bacteria. Distaso had very little clinical evidence to support his claim; however, it may have served as a conceptual foundation for today's research on lipopolysaccharides (LPSs).

In 1922, research first discovered what modern physicians would recognize as small intestinal bacterial overgrowth (SIBO). The physician who described it, Issac Jankelson, linked it to "neurasthenia." Neurasthenia was a term in the early twentieth century used to describe a cluster of modern day comorbidities like irritable bowel syndrome, chronic fatigue, myalgic encephalomyelitis, fibromyalgia, anxiety, and depressive disorders. In the late 1920s, physician and microbiologist Lloyd Arnold found that environmental stress, pathogenic bacteria, nutritional deficiencies, or drastic changes in diet could cause SIBO and intestinal permeability. He focused heavily on dietary effects and found that dietary factors were critically important when laboratory animals were infected with pathogens. Arnold was also one of the first physicians to consider how the paleolithic diet could affect microbiota and its effect on human survival.

Continuing in the 1920s and into the 1930s, more authors began to report on the effects of altered microbiota and intestinal intolerance for carbohydrates. This was either through direct alteration of microbiota or through reduced gastric acid production. The authors reported alternating constipation and diarrhea as well as anxiety as major presenting symptoms. They also reported improvement in symptoms with supplementation with acidophilus milk. Still other authors studying patients with mental disorders found that many (54%) demonstrated alterations in gastric acid production (as compared to 20% of healthy controls). Most of the patients demonstrated hypochlorhydria. It was concluded that because of the lack of hydrochloric acid in the stomach, bacteria that might otherwise be killed were allowed access to the rest of the GI tract, creating imbalances that eventually manifested as mood change.[7]

It was also during the early twentieth century that the contributions of diet to the microbiota balance began to be examined. In 1910, it was noted that diets high in protein increased proteolytic bacteria while reducing lactobacillus and Bifidobacterium species in cats and monkeys. Conversely, the opposite was true if animals were placed on a carbohydrate diet that also included milk. The animals in these experiments were also observed to show changes in cognition and mood. While study in the area of "autointoxication" and the microbiota-brain connection was popular in the early twentieth century, further studies conducted in the 1940s and 1950s were largely ignored and dismissed. Autointoxication fell out of favor, possibly because of the term itself. It was connected to just about every acute and chronic medical disorder of the day creating a high degree of skepticism in the medical community. Studies began to arise that contradicted the findings of the early twentieth century. This, combined with a healthy dose of skepticism and concern about newly marketed and unregulated treatments for autointoxication, doomed the brain-gut connection—at least for most of the rest of the century. There was a spark of interest in the 1960s when it was noted that antibiotics and *Lactobacillis acidophilus* could reduce cognitive symptoms associated with hepatic encephalopathy, but the interest was fleeting. With autointoxication "debunked," other theories rose to explain the connections between GI disturbance and cognitive change. Freud's theories were popular as were many other top-down explanations. The era of the somaticizing patient was upon medicine.[7]

Ultimately, poor explanation, a concept of panacea, charlatanism, and a greater interest in cognitive theories ended the autointoxication theory. Promising lines of research that could explain true gut-brain connectivity (intestinal permeability, LPS endotoxemia, probiotic strain investigation, cytokine changes) were impeded.[7] It would take another 70 years before the research would start to explore the gut-brain connection again.

"LEAKY" GUT

Given the historical perspective, it is easy to see why medicine has not received the concept of GI hyperpermeability with open arms. From a research standpoint, the concept was specifically

avoided for the better part of the twentieth century. However, new lines of research support the idea of hyperpermeability and effects it might have on brain function. In order to grasp the concept of the gut affecting the brain, one must first understand the concept of "leaky gut."

According to Groschwitz and Hogan, "The intestinal epithelium is a single-cell layer that constitutes the largest and most important barrier against the external environment."[14] This barrier is *selectively* permeable. It is important that it remains selectively permeable so nutrients are properly absorbed but toxins, enteric flora, and antigens are not.[14] The absorption of antigenic substances stimulates a potent immune response that may disrupt host inflammatory balance, ultimately leading to changes in cytokines, immunity, neurotransmitters, and synaptic plasticity. Before proceeding, it is important the reader understands what cytokines are. "Cytokines are nonantibody proteins released by cells on contact with antigens and that act as intercellular mediators."[15] They may be inflammatory, anti-inflammatory, or modulatory. When the immune system is stimulated, cytokines are released and communicate with the brain. In this way, the immune system acts as a sensory organ,[14] which may influence mood.

Permeability through the GI epithelial barrier is regulated through two routes: transcellular and paracellular. The transcellular route involves selective transporters for amino acids, electrolytes, short-chain fatty acids, and sugars for passage directly through epithelial cells. The paracellular pathway is associated with transport between cells. Tight junctions, desmosomes, and adherens junctions located at the apical and lateral borders of the cells regulate paracellular flow. In an ideal scenario both paracellular and transcellular permeability remain selective and appropriate. However, there are factors that alter permeability. In vitro and in vivo animal studies have shown exogenous factors (alcohol, NSAIDs, and physical and psychological stress), cytokine production (IFN-γ, IL-4, IL-13, and TNF-α), and immune cells (T cells, mast cells, and eosinophils) change intestinal permeability. Still other studies have shown microbial dysbiosis, high fructose diet, and certain nutrient deficiencies like vitamins A and D, magnesium, zinc, and calcium also result in increased intestinal permeability.[16] This change in GI permeability is currently postulated to be a predisposing and aggravating factor in many established autoimmune and inflammatory conditions including inflammatory bowel disease, food allergy, celiac disease, and diabetes.[14]

Of particular interest with respect to intestinal permeability and its effect on host mood is the concept of lipopolysaccharide (LPS) stimulation of the immune system. LPS translocation through the gut barrier (and subsequent elevations in serum) is commonly referred to as endotoxemia. LPSs are found on the surface of gram-negative bacteria and function to stabilize membrane integrity. They elicit a very strong immune response. By far the largest potential source of LPS in the human body is the gut. Under normal circumstances, exceptionally small amounts of LPS are found circulating in the blood. This would indicate that in a healthy person, the gut barrier does a reasonably good job keeping LPS in the gut lumen. In people with disorders known to be associated with inflammation like obesity and diabetes, the levels of circulating LPS are higher, suggesting a breakdown of the intestinal barrier.[17] Immune response to gut bacteria has also been demonstrated in patients with major depression. This suggests intestinal hyperpermeability. LPS translocated through an overly permeable intestinal barrier are capable of modifying the biochemistry of the CNS. This is through its ability to stimulate a strong immune response and, therefore, shift inflammatory load. The effects of LPS stimulation on the CNS are not entirely unknown. Fever is a very good example. Exposure to LPS induces an inflammatory cytokine response via IL-1β, IL-6, and TNF-α. In turn, this stimulates cyclooxygenase 2 (COX-2) to synthesize prostaglandin E2 (PGE2) in the CNS, resulting in fever.[18]

Qin showed that peripheral LPS stimulation in animals was associated with a significant increase in neuroinflammation. In this study, LPS or tumor necrosis factor alpha were injected into adult wild-type mice. LPS injection caused a rapid spike in brain TNF-α that persisted for an astounding 10 months. Conversely, TNF-α injection caused serum and liver levels to rise for only 9 hours and 1 week, respectively. In the end, both systemic administration of LPS and TNF-α caused microglia to activate brain pro-inflammatory mediators; however, LPS was far more powerful and long lasting.

Additionally, LPS stimulation caused progressive loss of dopamine neurons in the substantia nigra. The activation of glial cells is particularly concerning as research has demonstrated activation may become continuous and uncontrolled. The resulting neurotoxicity cannot be "turned off" even if the instigating stimulus is removed.[19] Although glial activation is a necessary and protective step in the brain, chronic activation is associated with increased brain inflammation. This glial activation, perhaps driven by LPS stimulation from a highly permeable gut, leads to a pro-inflammatory state in the brain. In a susceptible person, this may cause mood disturbance. In fact, LPS stimulation and consequential systemic inflammatory response are tied to major depression. A systemic inflammatory response causes pro-inflammatory cytokine production (such as IL-6, IL-1β, and TNF-α), increased markers of T-lymphocyte activation, decreased serum zinc levels, and an increased induction of indoleamine 2,3 dioxygenase.[18] This culminates in increased brain inflammation, reduced availability of tryptophan for serotonin production, blood-brain barrier breakdown, and further degradation of gut barrier integrity.

Major depression shares significant comorbidity with inflammatory disease. Confounding factors make it difficult to determine if the patient is depressed because of an inflammatory comorbidity, or if the depression and their systemic illness are both rooted in high inflammatory load. Evidence is growing that both are likely of inflammatory origin. It has been reported by some that increased serum levels of IgM and IgA against LPS correlate to symptoms of MDD like fatigue, GI disturbance, autonomic disturbance, and subjective feelings of infection.[20] High antibody titers to LPS indicate gut barrier breakdown. With this information the picture of depression as an inflammatory disorder rooted in poor gut barrier function becomes clearer.

In a landmark study published in 2001 it was shown that, indeed, it is possible to induce mood changes in human beings with endotoxins (LPS). Reichenberg et al. showed in a double blind, crossover study, an injection with *Salmonella abortus-equi* endotoxin was associated with transient increases in anxiety and depressed mood in otherwise healthy subjects. Additionally, it was found memory was adversely affected with both verbal and nonverbal memory scores significantly reduced. The cytokine changes that were noted included increases in TNF-α, IL-6, and IL-1 receptor antagonist. The changes in mood and memory were highly correlated with levels of cytokines. Interestingly, other than a mild increase in rectal temperature, there were no other physical symptoms associated with endotoxin injection.[21] The lack of changes in physical symptoms supports the concept of persistent, low-grade inflammation disrupting mood without overt physical illness. This is what has been referred to as "sickness behavior" or behavioral adaptations to systemic inflammation that may have been evolutionarily adaptive as a means to conserve energetic resources for recovery. This concept is supported by studies demonstrating continuous, low-dose endotoxin infusion (rather than bolus) as more likely representative of human low-grade inflammation because of a sustained inflammatory response.[22] This makes sense in the context of a leaky gut as the source of endotoxin. It is likely to be a slow, steady flow of LPS into systemic circulation causing increased inflammatory load over months or perhaps years.

A hyperpermeable intestinal barrier is not selective for LPS but may also permit transit of antigenic proteins and environmental toxins. Polychlorinated biphenyls are particularly offensive chemicals and are linked to risk for depressive symptoms. In addition, they themselves can break down the gut and blood-brain barrier.[17]

CAUSES OF LEAKY GUT

So the question is, how does one develop "leaky gut?" Factors like alcohol, NSAIDs, physical and psychological stress, cytokine production, and immune cells increase intestinal permeability. Still other studies have shown microbial dysbiosis, high fructose diet, the Western diet, and certain nutrient deficiencies like vitamins A and D, magnesium, zinc, and calcium also result in increased intestinal permeability. Additionally, unnatural foods such as those treated with herbicides and pesticides and genetically modified organisms (GMOs) have been shown to alter the GI environment.

Stress is a major factor in many peoples' lives. It also breaks down the gut barrier and results in a reduced ability to control gut inflammation. Stress is a potent activator of the sympathetic nervous system (SNS) and though the SNS seems to display mild anti-inflammatory properties in the gut, the parasympathetic system has very powerful anti-inflammatory effects. As an example, stimulation of the vagus nerve in laboratory animals has been shown to prevent endotoxin-induced shock by reducing pro-inflammatory cytokine production.[23] The natural push-pull of the autonomic system means that powerful SNS activation causes decreased PNS activation resulting in a net loss of inflammatory control in the gut leading to barrier breakdown. Chronic stress also downregulates production of secretory IgA. Secretory IgA (SIgA) is an antibody produced in mucosal linings and plays a major role in host immunity. SIgA helps prevent antigens in the gut from being able to stick to the walls of the intestines. If these antigens are not excreted, they are able to produce an inflammatory response that may break down the gut barrier. Together with the intestinal microbiota, SIgA contributes to maintaining intestinal inflammatory response within physiological limits. Downregulation of SIgA, associated with stress, can have negative repercussions on intestinal function and integrity. This can take the form of increased adhesion of pathogenic agents to the intestinal epithelium and/or an altered balance of inflammation leading to greater intestinal permeability.[24]

Another mechanism that may increase permeability is the Western diet. This diet is often high in fructose and unhealthy fats. Fructose has been implicated in insulin resistance, fatty liver, and metabolic syndrome. Some studies have shown that fructose may induce fatty liver directly through increasing bacterial toxin (LPS) translocation from the gut and increased endotoxemia.[25] The fatty liver induced by fructose can be ameliorated when combined with antibiotics, suggesting that fructose may alter intestinal permeability through dysbiosis and activation of the immune system.[16] Further supporting this is the information showing that in mice who have been genetically modified not to have a specific receptor for endotoxins (and therefore cannot generate an inflammatory response), called a toll-like receptor, fructose exposure does not cause fatty liver or insulin resistance.[26] Additionally, Western diets are often high in calories. High-calorie diets are associated with higher levels of plasma LPS.[16] Excessive carbohydrate and fat intake have both been shown to increase translocation of LPS out of the gut and into the bloodstream.[27] Still more studies have shown the Western diet is able to shift microbial balance to one less favorable and more likely to produce obesity and a greater inflammatory load.[16] This all culminates in breakdown of the gut barrier in a self-perpetuating cycle of inflammation.

Vitamin and mineral deficiencies may also lead to increased gut permeability. It is through these nutrients' ability to change cytoskeletal structure or expression of tight junction proteins that they may be involved in changes in gut barrier function. Retinoic acid plays a major role in the expression of genes related to epithelial barrier and tight junctions. Vitamin A status regulates the cellular availability of retinoic acid. It has been shown that a reduction in vitamin A adversely affects barrier function and tight junction expression.[28] Similarly, magnesium deficiencies have been shown to reduce tight junction expression and reduce *Bifidobacterium* content in the gut.[29] In animal models, zinc deficiency has been shown to directly break down tight junctions and/or sensitize barrier cells to external stressors capable of increasing gut permeability like alcohol. Eventually, this leads to the breakdown of the gut barrier through zinc dyshomeostasis.[30] Finally, the vitamin D receptor (VDR) is hugely important in mucosal barrier function. It preserves junctional complexes, stimulates epithelial cell renewal, and modulates the immune function associated with mucosal barriers.[16] Further, administration of vitamin D to experimental animals increases colonic epithelial cell resistance to injury.[31]

Dysbiosis is also a cause of leaky gut. Dysbiosis is a term that is used to describe an imbalance that may develop between the "good" bacteria in the gut and the "bad" bacteria.[32] From the moment the gut is colonized, the microbiota serve an important protective function as they drive the functional interactions of all the elements of the adaptive immune system. This must be done by altering function not only within mucosal associated lymphoid tissue such as Peyer's patches, but also through structures outside of the gut like extraintestinal lymphoid tissue. This would obviously imply that the microbiota must at the very least be able to communicate with structures outside of

the gut, or possibly even have access through the GI barrier. Any subsequent disruption of the balance of said microbiota may alter the permeability of the barrier.[33] As with all things in the body, homeostasis and balance are key. Microbial balance is determined by many factors, which can be separated into two categories: host genetic factors and environmental factors.[34]

At this time, genetic factors are not modifiable; however, changes in the environment occur frequently. The things that may alter microbial balance include diet, antibiotic use, psychological and physical stress, radiation, altered GI peristalsis,[35] and lifestyle factors like exercise, alcohol, and smoking. The environmental factors are capable of creating dysbiosis known to generate inflammation and are seen in inflammatory bowel disease.[34] A hallmark of inflammatory bowel disease is breakdown of intestinal barrier function, which is driven, in part, by microbiota changes. Although more study is necessary to understand the complex relationship between gut microbiota changes and gut permeability,[16] current knowledge of pathogens suggests a strong link. Common mechanisms of disruption of GI permeability include the aforementioned inflammation, altered fluid and electrolyte transport, and tight junction interference. Enteric pathogens may also create toxins and proteases, which damage cells, initiate apoptosis of epithelial cells, or disrupt cytoskeletal structure. *Vibrio cholerae* is known to disrupt barrier function by disrupting tight junctions, intestinal ion and fluid transport, and inflammatory response. Enteropathogenic *E. coli* (EPEC) is also capable of breaking down tight junctions and altering intestinal ion secretion. Finally, *Clostridium perfringens*, a common cause of food poisoning, also destabilizes tight junctions through a number of mechanisms.[14]

In the past year, there has been an exploration of the impact of herbicides such as Monsanto's RoundUp (glyphosate) on our gut microbiome. As it turns out, this chemical is very active in the disruption of beneficial bacteria via its impact on the "shikimate pathway" previously assumed not to exist in humans.

By imbalancing this flora, pesticides/herbicides also disrupt the production of essential amino acids such as tryptophan, a serotonin precursor, and promote production of p-cresol, a compound that interferes with metabolism of other "xenobiotics" or environmental chemicals, making the individual more vulnerable to their toxic effects. Even vitamin D3 activation in the liver may be negatively impacted by glyphosate's effect on liver enzymes, potentially explaining epidemic levels of deficiency.

There is also evidence that insecticidal toxins such as "Bt" are transferred into the blood of pregnant women and their fetuses, and that glyphosate herbicide transfers to breast milk.[36] Genetic modification of foods, in addition to guaranteeing exposure to pesticides and herbicides, confers risks of gene transference to human gut bacteria, even after a singular exposure.[37]

Most people think of ibuprofen as an innocuous, over-the-counter comfort for aches and pains. Some are so lulled into a sense of safety and efficacy, that they keep these pills in their purses and nightstands for even daily use. In addition to other known risks, its effects on the small and large intestine may be best summarized by Mäkivuokko et al., who state[38]: "The initial biochemical local sub-cellular damage is due to the entrance of the usually acidic NSAID into the cell via damage of the brush border cell membrane and disruption of the mitochondrial process of oxidative phosphorylation, with consequent ATP deficiency."

Resulting increases in permeability allow for luminal factors to access the immune system and to set off autoimmune and inflammatory processes. More recent evidence suggests that unbalanced gut bacteria set the stage for NSAID-induced permeability through neutrophil stimulation.[39] Damage from NSAID use is visible within 12–24 weeks of use[39]; however, more sensitive chemical testing reveals damage may develop as quickly as 2 weeks after beginning use with inflammation starting after just 3 days.[40] Recommending enteric-coated NSAIDs is not beneficial. It only shifts damage from the proximal GI tract to the distal areas.[40]

CYTOKINES AND BRAIN CHEMISTRY

With the knowledge that factors like stress, diet, microbiota changes, and other environmental factors may increase intestinal permeability leading to immune activation through LPS stimulation

and subsequent inflammatory cytokine production, the behavioral effects may be best explained through the communication between body and brain.

Cytokines may act on central sites where the blood-brain barrier is weak or may actually cause breakdown of the blood-brain barrier (BBB). They may be transported in by selective transporters, therefore bypassing the BBB. Cytokines may act on peripheral nerves that send information into the CNS causing changes in feedback to the brain. Cytokines can affect the secretion of molecules that are not limited by the BBB but can themselves affect neurochemistry. Finally, immune cells that have infiltrated the CNS through an already compromised BBB can synthesize them.[40]

It has been shown that IL-1 has the capability of altering catecholamines. In experiments in rats, the injection of IL-1 increased the catabolites of norepinephrine (NE). This, of course, suggests there was an increased release of NE in response to IL-1. This response was seen globally in the brain; however, the greatest response was seen in the hypothalamus. Others have reported changes in dopamine metabolism in response to IL-1 injection; however, the data here are less consistent. Injection of IL-1β into rats in the first few days of life actually results in a permanent decrease in dopamine in the hypothalamus and supercervical ganglion. This occurs at surprisingly low doses. The mechanism by which IL-1 can create neurochemical changes appears to be through the cyclooxygenase (COX) enzymes. COX inhibitors prevent increases in 3-methoxy-4-hydroxyphenyl(ethylene)glycol (MHPG), which is a major metabolite of NE. In an interesting connection with the gut, abdominal vagotomy reduces increases in hypothalamic NE stimulated by IL-1β. This suggests the vagus may be important for proper modulation of immune response and proper neurochemical response.[41]

IL-1 may also affect serotonin and tryptophan metabolism. Interestingly, several experiments have shown that IL-1 administration increases brain tryptophan and 5-hydroxyindoleacetic acid (5-HIAA), the major catabolite of serotonin. This was not region specific. In the case of serotonin metabolism, COX inhibitors did not change the increase in tryptophan or 5-HIAA seen in the brain. Additionally, subdiaphragmatic vagotomy did not alter the serotonergic response to IL-1. According to some authors, increases in brain tryptophan and 5-HIAA are mediated by the sympathetic system because these changes can be prevented with ganglionic blockers. Therefore, they conclude that the increases in brain tryptophan reflect sympathetic activity.[41] The idea of IL-1 *increasing* brain tryptophan and 5-HIAA seems contradictory to current knowledge about depression and the serotonin hypothesis, so the question becomes, do the increased levels of 5-HIAA reflect increased turnover of serotonin? This makes sense in the context of depression, as increased serotonin turnover and mood disorder are associated.[42] Additionally, those who are depressed have demonstrated in studies higher levels of pro-inflammatory cytokines. These include IL-1, IL-6, and tumor necrosis factor.[43,44] In addition to affecting catecholamine and serotonin metabolism, IL-1 affects other neurotransmitters. Reductions in acetylcholine, glutamate, and gamma-aminobutyric acid (GABA) have been demonstrated as well as increases in histamine turnover.[41]

Interleukin-6 (IL-6) has been studied with respect to NE metabolism with no effects noted. However, changes in the HPA axis are noted in animal models as measured by plasma ACTH and corticosterone. Some authors have demonstrated IL-6 to increase 3,4-dihydroxyphenylacetic acid (DOPAC), a major metabolite of dopamine. This was particularly prevalent in the prefrontal cortex. These same authors also noted an increase in 5-HIAA in the hippocampus and prefrontal cortex.[41] Again, this may represent increased turnover of serotonin. Interestingly, IL-6 has also been demonstrated to increase serotonin itself in the hippocampus.[41] Perhaps this is related to the endocrine response to IL-6 and may not be relevant in mood disorder. More study is necessary.

Tumor necrosis factor-α (TNF-α) also activates the HPA axis, although IL-1 seems to be the most potent. TNF-α has been reported to increase brain MHPG and tryptophan, but only at very high doses. However, sensitization to TNF-α appears to be a phenomenon worth considering. Successive exposures to TNF-α produced marked enhancement in MHPG accompanied by changes in behavior such as decreases in activity and social exploration.[41]

Interferon-γ (IFNγ) has considerable effects on the metabolism of tryptophan and therefore can affect levels of serotonin in the brain. IFNγ has the capability to activate an enzyme called indoleamine-2,3-dioxygenase (IDO). IDO is found throughout the body and in human immune cells including macrophages and microglia. The vast majority of circulating tryptophan is metabolized by an enzyme called tryptophan-2,3-dioxygenase (TDO), where it is converted into kynurenine and other metabolites with the pathway eventually ending in niacin synthesis. This pathway is effective with less than 1% of circulating tryptophan being available for conversion to serotonin in the brain.[45] Under normal conditions, TDO is the dominant enzyme, however, with immune stimulation IDO can be activated. This has the effect of reducing serum tryptophan by as much as 25%–50%. This is important because the rate-limiting enzyme in serotonin production is tryptophan hydroxylase. It is only 50% saturated under normal circumstances, and therefore, serotonin synthesis varies with tryptophan availability.[45]

One can see how any reduction in serum tryptophan might present a problem. Although IFNγ is probably the most potent activator of IDO, other cytokines may activate it as well. TNF-α in combination with IL-6 or IL-1 can induce IDO, perhaps creating an additive effect of reducing tryptophan concentrations even further.[45] A human model of this phenomenon is seen in patients with hepatitis C (HCV). Patients with chronic HCV are often treated with long-term interferon therapy. Interferons also have the potential to activate IDO, albeit at a lower level.[41] Patients with HCV are treated with interferons for periods of 6–12 months. During the treatment period, up to a third of patients will report symptoms of depression (via standardized depression scales but self-reporting of depression results in even higher rates). The interferons administered increases inflammatory cytokine profiles, similar to those seen in depression. Although interferons are administered peripherally, central levels of inflammatory cytokines have been observed to rise. This is evidence of central communication. Also noted in HCV patients treated with interferons is an increase in the kynurenine:tryptophan ratio in the blood and cerebrospinal fluid (CSF). This suggests more tryptophan is being converted into kynurenine, making less available for conversion into serotonin. It also demonstrates increased activity of IDO in response to pro-inflammatory cytokine exposure.

Now that it is clear that cytokines can change neurochemistry, what about neurochemical responses to endotoxins? As it turns out, there is evidence of that as well. Administration of LPS decreases brain levels of NE; however, serotonin is not affected. Other evidence has shown small changes in dopamine metabolism to LPS stimulation. Levels of tryptophan and 5-HIAA have been shown to increase in response to LPS, perhaps more evidence of increased serotonin turnover. An experiment in rats demonstrated increases in extracellular DOPAC, NE, MHPG, and 5-HIAA after LPS injection.[41] Clearly, whether the mechanism affecting brain chemistry is through cytokine stimulation, direct LPS stimulation, or both, an effect is observed, and addressing this in the depressed patient might prove therapeutically valuable.

GUT-BRAIN COMMUNICATION

It is becoming quite clear that a bidirectional relationship exists between the human GI tract and the rest of the body, including the central nervous system. As with all bidirectional relationships, functional integrity of each system is required for optimal performance. A major part of this bidirectional system is the microbiota.

MICROBIAL EFFECTS ON THE CNS

The effects of microbial balance on CNS function begin early in life. The HPA axis is a neuroendocrine system. It may be thought of as a system that is highly subject to changes in the environment and is highly programmable. It has been shown that HPA response is different in animals that are handled and cared for as neonates versus animals who experience maternal deprivation. Maternal deprivation causes exaggerated HPA response to stress, and this response exists throughout the life

of the animal. This altered HPA response is associated with the incidence of age-related neuropathology. It has been speculated that because of the bidirectional relationship early in life between neural and immune systems at a time when the CNS is particularly susceptible to environmental influences, microbial colonization and subsequent effects on the immune system might alter the development of HPA responsiveness. This has been demonstrated in animals, with genetically engineered germ-free mice displaying exaggerated HPA response to mild stress. This response can be corrected by colonization from pathogen-free controls in the experiment. Additionally, it was shown that colonizing the germ-free mice with enteropathogenic *E. coli* could actually increase the stress response. This suggests that not only is the presence of commensal bacteria critical, but the absence of pathogens may be as well. Another important finding from the study was a reduction of brain-derived neurotrophic factor (BDNF) and reduced protein levels in the cortex and hippocampus of germ-free mice compared to pathogen-free mice. This is an important factor because BDNF is involved in many regulatory processes in the brain, including mood and cognition.[46] In addition, levels of norepinephrine and serotonin have been shown to be reduced in germ-free animals. This was noted in both the hippocampus and cortex.[47] Conclusions can be made that postnatal colonization may play a significant role in early development and can have long-lasting impacts on sensory and neural processing of information as it relates to endocrine response to stress.[46] This information is quite suggestive, and most authors are showing that there is a critical window for colonization of the gut. After this window, the addition of bacteria from pathogen-free mice is not effective in reducing the stress response. If nothing else, this has implications on the high rate of cesarean births in the United States.

Changes in HPA axis function are well documented in depression. The connection is so strong that some authors consider it to be a causal factor. Elevated serum cortisol and CSF corticotropin-releasing hormone is well documented in the depressed patient. Stress is also linked to changes in the HPA axis. As reviewed previously, stress also affects gut immunity and gut barrier integrity. Stress has another effect. It has been shown to alter the microbiome. It can promote the growth of pathogenic *E. coli* O157:H7.[47] The brain itself has been shown to be responsive to the introduction of bacteria into the GI tract. This connection is thought to be from an immune response communicated to the brain via the vagus nerve. In one experiment, *Salmonella* Typhimurium (STM) was introduced into the stomach of mice to mimic an infection. STM also infects humans. STM-induced activation of the paraventricular nucleus and the supraoptic nucleus was seen,[48] and the introduction of bacteria to mice has been shown to produce anxiety-like behavior.[49] The question remains, does the stress alter the bacterial profile significantly enough to change CNS signaling, or does microbial overgrowth increase perceived stress? A combination of both is likely.

Still other evidence has shown gut microbiota can influence not only visceral pain perception (an easy connection to make) but also somatic pain. This information is further support for a connection between the microbiome and the CNS.[47]

Animal studies support the theory that microbiota can affect behavior. In mice, subclinical oral administration of *Campylobacter jejuni* produced anxiety. Perhaps more interesting is that the brainstem was also shown to be active in these animals. The communication line is likely the vagus. The information sent to the CNS from a stimulus like *Campylobacter jejuni* ultimately leads to autonomic, neuroendocrine, and behavioral change. Other studies have shown that dietary-induced changes designed to increase the diversity of the microbiome had a positive effect on memory and reduced anxiety-like behavior.[47]

The next logical step then is to understand just how microbial changes may be able to directly affect the CNS. It is well accepted that the commensal organisms present in the gut produce serotonin, melatonin, GABA, catecholamines, histamine, and acetylcholine. Although these neurotransmitters do not cross the blood-brain barrier in any appreciable amount, they may alter visceral reflexes which may affect CNS function. One interesting theory is that these neurotransmitters are produced so that bacteria may communicate with the host—so called "inter-kingdom signaling." Further, *Lactobacilli* have been shown to increase the activity of IDO, further altering tryptophan metabolism.[47]

The mechanism of communication may be through short-chain fatty acids (SCFAs). SCFAs "are the end products of anaerobic bacterial fermentation in the GI tract. Under physiological conditions, their production is entirely dependent on commensal microbes" (p. 13).[47] A major SCFA is butyric acid. Bacteria such as *Clostridium* species produce it. Studies have shown that injection of butyrate produces changes in the hippocampus and frontal cortex and displays antidepressant effects. One factor noted was an increase in BDNF in the frontal cortex of the mice in the experiment. Butyrate also has large effects locally in the GI tract, so this may be the mechanism through which it affects the host's mood and behavior. Other SCFAs may also have effects. Propionic acid has also been shown in experiments to create behavioral change.[47]

Evidence linking omega-3s with reduced clinical presentation in various psychiatric disorders abounds. While this link remains controversial, the mechanism may actually be through commensal bacteria. Mice fed a diet containing fish oil had a threefold increase in bifidobacteria and reduced quantities of *Bacteroides.* The connection may be through the ability of omega-3s to alter SCFA production.[47]

BENEFICIAL EFFECTS OF PROBIOTICS

Probiotics have long been known to have beneficial effects as noted by the historical perspectives section of this chapter. The exact mechanisms by which they may affect mood are less clear, but are likely to be mechanistically related to cytokine production and immune/inflammatory activation. The term *probiotic* has been defined as a live organism that, when ingested in adequate amounts, exerts a health benefit. Many bacteria are purported to be probiotic; however, few have actually been studied. Recently, a new term has been introduced in the literature—*psychobiotic.* The authors defined this as a live organism that, when ingested in adequate amounts, produces a health benefit in patients suffering from psychiatric illness. Clinical studies have been performed with promising results. Irritable bowel syndrome (IBS) is a common disorder that is known to include disturbances of the gut-brain axis. Depression and anxiety are not uncommon in patients with IBS. Placebo-controlled trials comparing *B. infantis* and *L. Salivarius* show that the former significantly improves all symptoms. Still other placebo-controlled trials have shown the combination of *L. helveticus* R0052 and *B. longum* reduced psychological stress as measured by the Hopkins Symptom Checklist, the Hospital Anxiety and Depression Scale, and the Coping Checklist. This study also showed a reduction in urinary free cortisol levels. Elevated cortisol is frequently seen in depression.[50] Probiotic-containing yogurt has even shown to be beneficial when compared to placebo after 3 weeks in a self-reported experiment. Finally, in patients with chronic fatigue syndrome who were given a yogurt drink containing *Lactobacillus casei* or a placebo, those receiving the probiotic had significant improvements in anxiety.[50]

BUILDING A HEALTHY MICROBIOME

Building and maintaining a healthy microbiome is essential to optimizing brain function. Building a healthy microbiome begins before one even has control of it. With the knowledge that microbes likely inhabit the placenta, umbilical cord and fetal membranes, and amniotic fluid,[51,52] it becomes clear that the overuse of antibiotics is of major concern. Cesarean section rates in the United States might also be of concern.

According to the Centers for Disease Control and Prevention (CDC), one-third of all births in the United States are done by cesarean section. This is a sterile birth. During vaginal delivery, the contact with maternal vaginal and intestinal flora is an important source for the start of the infant's colonization. During cesarean delivery, this direct contact is absent, and nonmaternally derived environmental bacteria play an important role for infants' intestinal colonization.[52] Evidence has shown mode of delivery to affect colonization in infants. Babies born vaginally were colonized predominantly by *Lactobacillus*, whereas cesarean delivery (CD) babies were colonized

by a mixture of potentially pathogenic bacteria typically found on the skin and in hospitals, such as *Staphylococcus* and *Acinetobacter, s*uggesting babies born by CD were colonized with skin flora in lieu of traditionally vaginal types of bacterium.[52]

Differences in the microbiome between babies born vaginally and via cesarean section have been demonstrated to be present anywhere from 6 months postpartum to 7 years. Clinical relevance to these differences is scant. However, there are epidemiological studies showing possible changes in physiology as it relates to mode of delivery. It is well established that intestinal bacteria play an important role in the developing immune system. Atopic diseases appear more often in infants after cesarean delivery than after vaginal delivery.

Additionally, diseases like allergic rhinitis, asthma, celiac disease, diabetes mellitus type 1, and gastroenteritis are also more likely with cesarean delivery. Interestingly, the risks for allergic rhinitis and asthma go up with repeat cesarean.[52] Given this evidence and experimental animal evidence of changes to HPA axis function and stress response, these changes likely have longer-lasting influences on mood stability as well. Another critically important window for building a healthy microbiome is breastfeeding. In addition to direct maternal contact, breast milk itself acts as a stimulator for proper microbiome development. As some studies have shown delayed lactation in cesarean births, both the sterility of cesarean birth and delayed lactation might have long-lasting adverse effects on fetal microbiome development.[52] Breast milk is the perfect microbiome-starter. It is much more than protein, carbohydrate, and fat. It contains immune factors, bacteria (termed *entero-mammary transfer*[53]), and over 200 unique oligosaccharides designed to help build and maintain the microbiome. Patients and doctors should strive for low intervention births with breastfeeding being encouraged immediately after birth.

Given this information, the use of antibiotics during pregnancy also requires examination. In particular, treatment for group B *Streptococcus* needs a closer look. A review from the *Cochrane Database of Systematic Reviews* stated, "This review finds that giving antibiotics is not supported by conclusive evidence. The review identified four trials involving 852 GBS positive women. Very few of the women in labor who are GBS positive give birth to babies who are infected with GBS and antibiotics can have harmful effects such as severe maternal allergic reactions, increase in drug-resistant organisms and exposure of newborn infants to resistant bacteria, and postnatal maternal and neonatal yeast infections" (p. 2).[54] Given the effects of a mother's microbiota on a baby's, a reexamination of this practice is necessary.

After the earliest years, other factors play a significant role in keeping the microbiome healthy. Exercise is a critical factor. Increased exercise levels are associated with an increased diversity of the microbiome.[55] A study in rats found similar results, concluding that "Exercise was shown to enhance the relative abundance of three genera, with Lactobacillus being the most abundant, while another three genera were shown to be more abundant before exercise training (*Streptococcus*, *Aggregatibacter* and *Sutterella*)."[56] Similarly, another study in rodents showed *Lactobacillus* and *Bifidobacterium* (among others) were increased in an exercise group as compared to controls. The researchers concluded that exercise was an important factor in determining diversity of the microbiome. Additionally*, Lactobacillus* and *Bifidobacterium* have the capacity to produce the organic acid lactate, which is converted into butyrate, an important regulatory short-chain fatty acid in the gut.[57]

Since diet is perhaps the most modifiable aspect of human health, it requires consideration in maintaining a healthy microbiome. Prebiotic compounds are a logical place to start. Prebiotics can be defined as food ingredients that "selectively stimulate the growth and/or activity of those bacteria that contribute to colonic and host health" (p. 1418).[58] Prebiotics, or nondigestable oligosaccharides, are of very little caloric value but enhance mineral absorption, lower the risk of infection and diarrhea, modulate the immune system, and favorably impact microbiota. The major dietary source of prebiotics is fructans, including chicory root extract, inulin, oligofructose, and short-chain fructooligosaccharides. Prebiotics work mostly by stimulating growth of beneficial bacteria in the gut. The consumption of prebiotics has been demonstrated to increase bifidobacteria and lactobacilli as well as butyrate producers like *Eubacterium*, *Faecalibacterium*, and *Roseburia*. Prebiotics have

also been shown to increase total short-chain fatty acid content. However, more study is necessary in this area.[59]

In addition to prebiotic consumption, our food choices matter a great deal. The high-fat/high-sugar Western diet alters the genetic composition and metabolic activity of the microbiome. We must also consider these diets are generally also very low in fiber. These changes are now being recognized as contributing to the ever-increasing chronic disease burden. Evidence in mice has shown that changes in macronutrient content can change gut microbiota in just one day.

In humans, this process has been demonstrated to occur over weeks to months in some experimental models. A more recent experiment has shown that, yes indeed, human gut microbiota responds within days as well.[60] What does the change in microbial content look like? High-carbohydrate diets, regardless of the glycemic index of that diet increase saccharolytic bacteria. High-fat diets reduce bacterial numbers while increasing the excretion of short-chain fatty acids.[61]

Perhaps more important than the macronutrient distribution of the diet is the fiber intake. A study comparing fecal microbiota of European children and that of children from a rural African village of Burkina Faso is an interesting comparison. Diets in this African village are high in fiber content, similar to that of early human settlements at the time of the birth of agriculture. The differences were striking. The African children showed enrichment in *Bacteroidetes* and depletion in *Firmicutes*. Also, bacteria from the genera *Prevotella* and *Xylanibacter* were present in much greater quantity compared to in European children. In fact, these were completely lacking in European children. This is important because *Prevotella* and *Xylanibacter* are important for breaking down plant starches. Short-chain fatty acids were also significantly more prevalent in African children. The study authors "hypothesize that gut microbiota coevolved with the polysaccharide-rich diet of BF individuals, allowing them to maximize energy intake from fibers while also protecting them from inflammation and noninfectious colonic diseases."[62]

Another characterization of the ancestral microbiome has been attempted by Schnorr et al.[63] who assessed the fecal microbiota of 27 Hazda hunter-gatherers, among the last remaining traditional cultures who rely exclusively on foraged plants, berries, and tubers as well as hunted game. They compared the phylogenetic diversity, taxonomic relative abundance, and short-chain fatty acid profile of these samples to those of 16 urban Italians eating a classical Mediterranean diet. The researchers found significant variation between the two community samples, most notably that the Hazda microbiome was dominated by *Firmicutes*, that 22% of the bacteria were as of yet unclassified, and that *Bifidobacteria* was notably absent. There were also significant gender differences including female Hazda samples notable for *Treponema* strains, considered to be opportunistic or pathogenic, suspected to aid in degradation of the relatively higher tuber intake. The fatty acids produced by Hazda microbiota were primarily propionate (rather than butyrate), which travels to the liver for gluconeogenesis (sugar formation). The Hazda samples also bore more resemblance to other catalogued African rural samples than to Western samples.

Authors of this study stress the need for thorough understanding of the presence of important confounding variables regarding one's diet such as the growth location, method of harvesting, and manufacturing processes. The role of soil appears paramount. Soil within the United States lacks many vital nutrients,[64] due in part to harmful industrialization of agriculture practices that interfere with natural ecosystems, supporting plants rich in nutrients. Similarly, fungicides and biocides have decimated the microbial ecology of the soil[65] in ways that fundamentally derail plant-based communication about the botanical environment. Plants are a dynamic reflection of their own microbiomes,[66] passing vital information about shared environments onto human consumers.

The Western diet, which is generally too rich in all macronutrients and too low in indigestible fibers, significantly contributes to an unfavorable microbiome and is associated with the rapid increase in noninfectious intestinal disease.[60] All of these diseases demonstrate a high degree of inflammation making it possible that this is partly the mechanism through which the Western diet contributes to chronic disease, including mood disorder. Diets rich in fiber and calorically calibrated appear to be most beneficial for building and maintaining a healthy microbiome. In practice,

recommending a paleolithic-inspired diet rich in vegetables and protein and low in high glycemic fruits and grain is a practical approach.

CONCLUSION

The interconnectedness of the gut, brain, immune, and hormonal systems is exceedingly difficult to unwind. Until we begin to appreciate this complex relationship, we will not be able to prevent or intervene effectively in depression, slated to become the second-leading cause of disability in this country, within the decade. For true healing and meaningful prevention, it is advisable for patients to take steps every day toward sending their body the message that it is not being attacked, it is not in danger, and it is well nourished, well supported, and calm.

As a society, we can begin to think about protecting the microbiome by demedicalizing birth and infant nutrition, and as individuals, by avoiding antibiotics, NSAIDs, gluten-containing grains, and genetically modified and nonorganic food. Promising interventions for depression from a gut-brain perspective include probiotics, fermented foods as part of a high natural fat diet, and relaxation response for optimal digestion, anti-inflammatory, and insulin sensitizing effects. This is termed *psychoneuroimmunology*, and likely represents the future of mental health care, compelling clinicians and researchers alike to appreciate the connectedness of different bodily systems, as well as our connectedness as a species to the environmental ecosystem in and around us.

REFERENCES

1. Kirsch, I. and Sapirstein, G. 1998. Listening to Prozac but hearing placebo: A meta-analysis of antidepressant medication. *Am Psychol Assoc* 1(2): 1–16.
2. Moncrieff, J. and Kirsch, I. 2005. Efficacy of antidepressants in adults. *BMJ* 331(7509): 155–157. doi:10.1136/bmj.331.7509.155.
3. Kirsch, I., Deacon, B.J., Huedo-Medina, T.B. et al. 2008. Initial severity and antidepressant benefits: A meta-analysis of data submitted to the Food and Drug Administration. *PLoS Med* 5(2): e45. doi:10.1371/journal.pmed.0050045.
4. Littrell, J.L. and Lacasse, J.R. 2012. The controversy over antidepressant drugs in an era of evidence-based practice. *Soc Work Ment Health* 10(6): 445–463. doi:10.1080/15332985.2012.699444.
5. Goldberg, D., Privett, M., Ustun, B. et al. 1998. The effects of detection and treatment on the outcome of major depression in primary care: A naturalistic study in 15 cities. *Br J Gen Pract* 48(437): 1840–1844.
6. Van Weel-Baumgarten, E.M., van den Bosch, W.J., Hekster, Y.A. et al. 2000. Treatment of depression related to recurrence: 10-year follow-up in general practice. *J Clin Pharm Ther* 25(1): 61–66.
7. Bested, A.C., Logan, A.C., and Selhub, E.M. 2013. Intestinal microbiota, probiotics and mental health: From Metchnikoff to modern advances: Part I—Autointoxication revisited. *Gut Pathogen* 5(1): 5.
8. Grenham, S., Clarke, G., Cryan, J.F. et al. 2011. Brain-gut-microbe communication in health and disease. *Front Physiol* 2: 94.
9. Mshvildadze, M., Neu, J., Shuster, J. et al. 2010. Intestinal microbial ecology in premature infants assessed with non-culture-based techniques. *J Pediatr* 156(1): 20–25.
10. Aagaard, K., Ma, J., Antony, K.M. et al. 2014. The placenta harbors a unique microbiome. *Sci Transl Med* 6(237): 237ra65.
11. Palmer, C., Bik, E.M., DiGiulio, D.B. et al. 2007. Development of the human infant intestinal microbiota. *PLoS Biol* 5(7): e177.
12. Frank, D.N., and Pace, N.R. 2008. Gastrointestinal microbiology enters the metagenomics era. *Curr Opin Gastroenterol* 24(1): 4–10.
13. Kurokawa, K., Itoh, T., Kuwahara, T. et al. 2007. Comparative metagenomics revealed commonly enriched gene sets in human gut microbiomes. *DNA Res* 14(4): 169–181.
14. Groschwitz, K.R. and Hogan, S.P. 2009. Intestinal barrier function: Molecular regulation and disease pathogenesis. *J Allergy Clin Immunol* 124(1): 3–20.
15. Yirmiya, R. 2000. Depression in medical illness: The role of the immune system. *West J Med* 173(5): 333–336.
16. Teixeira, T.F., Collado, M.C., Ferreira, C.L. et al. 2012. Potential mechanisms for the emerging link between obesity and increased intestinal permeability. *Nutr Res* 32(9): 637–647.

17. Bested, A.C., Logan, A.C., and Selhub, E.M. 2013. Intestinal microbiota, probiotics and mental health: From Metchnikoff to modern advances: Part II—Contemporary contextual research. *Gut Pathogen* 5(1): 3.
18. Vojdani, A. and Lambert, J. 2011. Functional neurology and immunology: VI. Crossing Barriers—Gut-to-brain lessons from interdisciplinary collaboration. *Funct Neurol Rehabil Ergon* 1(4): 631–644.
19. Qin, L., Wu, X., Block, M.L. et al. 2007. Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration. *Glia* 55(5): 453–462.
20. Maes, M., Kubera, M., and Leunis, J.C. 2008. The gut-brain barrier in major depression: Intestinal mucosal dysfunction with an increased translocation of LPS from gram negative enterobacteria (leaky gut) plays a role in the inflammatory pathophysiology of depression. *Neuro Endocrinol Lett* 29(1): 117–124.
21. Reichenberg, A., Yirmiya, R., Schuld, A. et al. 2001. Cytokine-associated emotional and cognitive disturbances in humans. *Arch Gen Psychiatry* 58(5): 445–452.
22. Taudorf, S., Krabbe, K.S., Berg, R.M. et al. 2007. Human models of low-grade inflammation: Bolus versus continuous infusion of endotoxin. *Clin Vaccine Immunol* 14(3): 250–255.
23. Vida, G., Peña, G., Deitch, E.A. et al. 2011. α7-cholinergic receptor mediates vagal induction of splenic norepinephrine. *J Immunol* 186(7): 4340–4346.
24. Campos-Rodríguez, R., Godínez-Victoria, M., Abarca-Rojano, E. et al. 2013. Stress modulates intestinal secretory immunoglobulin A. *Front Integr Neurosci* 7: 86.
25. Bergheim, I., Weber, S., Vos, M. et al. 2008. Antibiotics protect against fructose-induced hepatic lipid accumulation in mice: Role of endotoxin. *J Hepatol* 48(6): 983–992.
26. Spruss, A., Kanuri, G., Wagnerberger, S. et al. 2009. Haub S, Bischoff SC, Bergheim I. Toll-like receptor 4 is involved in the development of fructose-induced hepatic steatosis in mice. *Hepatology* 50(4): 1094–1104.
27. Amar, J., Burcelin, R., Ruidavets, J.B. et al. 2008. Energy intake is associated with endotoxemia in apparently healthy men. *Am J Clin Nutr* 87(5): 1219–1223.
28. Osanai, M., Nishikiori, N., Murata, M. et al. 2007. Cellular retinoic acid bioavailability determines epithelial integrity: Role of retinoic acid receptor alpha agonists in colitis. *Mol Pharmacol* 71(1): 250–258.
29. Pachikian, B.D., Neyrinck, A.M., Deldicque, L. et al. 2010. Changes in intestinal bifidobacteria levels are associated with the inflammatory response in magnesium-deficient mice. *J Nutr* 140(3): 509–514.
30. Zhong, W., McClain, C.J., Cave, M. et al. 2010. The role of zinc deficiency in alcohol-induced intestinal barrier dysfunction. *Am J Physiol Gastrointest Liver Physiol* 298(5): G625–G633.
31. Kong, J., Zhang, Z., Musch, M.W. et al. 2008. Novel role of the vitamin D receptor in maintaining the integrity of the intestinal mucosal barrier. *Am J Physiol Gastrointest Liver Physiol* 294(1): G208–G216.
32. Tamboli, C.P., Neut, C., Desreumaux, P. et al. 2004. Dysbiosis in inflammatory bowel disease. *Gut* 53(1): 1–4.
33. Anders, H.J., Andersen, K., and Stecher, B. 2013. The intestinal microbiota, a leaky gut, and abnormal immunity in kidney disease. *Kidney Int* 83(6): 1010–1016.
34. Doré, J., Simrén, M., Buttle, L. et al. 2013. Hot topics in gut microbiota. *United European Gastroenterol J* 1(5): 311–318.
35. Hawrelak, J.A. and Myers, S.P. 2004. The causes of intestinal dysbiosis: A review. *Altern Med Rev* 9(2): 180–197.
36. Aris, A. and Leblanc, S. 2011. Maternal and fetal exposure to pesticides associated to genetically modified foods in Eastern Townships of Quebec, Canada. *Reprod Toxicol* 31(4): 528–533.
37. Samsel, A. and Seneff, S. 2013. Glyphosate's suppression of cytochrome P450 enzymes and amino acid biosynthesis by the gut microbiome: Pathways to modern diseases. *Entropy* 15: 1416–1463.
38. Mäkivuokko, H., Tiihonen, K., Tynkkynen, S. et al. 2010. The effect of age and non-steroidal anti-inflammatory drugs on human intestinal microbiota composition. *Br J Nutr* 103(2): 227–234.
39. Sigthorsson, G., Tibble, J., Hayllar, J. et al. 1998. Intestinal permeability and inflammation in patients on NSAIDs. *Gut* 43(4): 506–511.
40. Tacheci, I., Kopacova, M., Rejchrt, S. et al. 2010. Non-steroidal anti-inflammatory drug induced injury to the small intestine. *Acta Medica (Hradec Kralove)* 53(1): 3–11.
41. Dunn, A.J. 2006. Effects of cytokines and infections on brain neurochemistry. *Clin Neurosci Res* 6(1–2): 52–68.
42. Barton, D.A., Esler, M.D., Dawood, T. et al. 2008. Elevated brain serotonin turnover in patients with depression: Effect of genotype and therapy. *Arch Gen Psychiatry* 65(1): 38–46.
43. Owen, B.M., Eccleston, D., Ferrier, I.N., and Young, A.H. 2001. Raised levels of plasma interleukin-1β in major and postviral depression. *Acta Psychiatr Scand* 103(3): 226–228.

44. Levine, J., Barak, Y., Chengappa, K.N. et al. 1999. Cerebrospinal cytokine levels in patients with acute depression. *Neuropsychobiology* 40(4): 171–176.
45. Christmas, D.M., Potokar, J., and Davies, S.J. 2011. A biological pathway linking inflammation and depression: Activation of indoleamine 2,3-dioxygenase. *Neuropsychiatr Dis Treat* 7: 431–439.
46. Sudo, N., Chida, Y., Aiba, Y. et al. 2004. Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. *J Physiol* 558(1): 263–275.
47. Forsythe, P., Sudo, N., Dinan, T. et al. 2010. Mood and gut feelings. *Brain Behav Immun* 24(1): 9–16.
48. Wang, X., Wang, B.R., Zhang, X.J. et al. 2002. Evidences for vagus nerve in maintenance of immune balance and transmission of immune information from gut to brain in STM-infected rats. *World J Gastroenterol* 8(3): 540–545.
49. Collins, S.M., and Bercik, P. 2009. The relationship between intestinal microbiota and the central nervous system in normal gastrointestinal function and disease. *Gastroenterology* 136(6): 2003–2014.
50. Dinan, T.G., Stanton, C., and Cryan, J.F. 2013. Psychobiotics: A novel class of psychotropic. *Biol Psychiatry* 74(10): 720–726.
51. Funkhouser, L.J. and Bordenstein, S.R. 2013. Mom knows best: The universality of maternal microbial transmission. *PLoS Biol* 11(8): e1001631.
52. Neu, J. and Rushing, J. 2011. Cesarean versus vaginal delivery: Long-term infant outcomes and the hygiene hypothesis. *Clin Perinatol* 38(2): 321–331.
53. Jost, T., Lacroix, C., Braegger, CP. et al. 2014. Vertical mother-neonate transfer of maternal gut bacteria via breastfeeding. *Environ Microbiol* 16(9): 2891–2904.
54. Ohlsson, A. and Shah, V.S. 2014. Intrapartum antibiotics for known maternal Group B streptococcal colonization. *Cochrane Database Syst Rev* 2014(6): 1–43.
55. Ray, K. 2014. Gut microbiota. Tackling the effects of diet and exercise on the gut microbiota. *Nat Rev Gastroenterol Hepatol* 11(8): 456.
56. Petriz, B.A., Castro, A.P., Almeida, J.A. et al. 2014. Exercise induction of gut microbiota modifications in obese, non-obese and hypertensive rats. *BMC Genomics* 15: 511.
57. Queipo-Ortuño, M.I., Seoane, L.M., Murri, M. et al. 2013. Gut microbiota composition in male rat models under different nutritional status and physical activity and its association with serum leptin and ghrelin levels. *PLoS One* 8(5): e65465.
58. Slavin, J. 2013. Fiber and prebiotics: Mechanisms and health benefits. *Nutrients* 5(4): 1417–1435, doi:10.3390/nu5041417.
59. Institute of Medicine (US) Food Forum. 2013. *The Human Microbiome, Diet, and Health: Workshop Summary*. Washington, DC: National Academies Press; 5, Influence of Diet and Dietary Components on the Microbiome. Available from: http://www.ncbi.nlm.nih.gov/books/NBK154087/
60. David, L.A., Maurice, C.F., Carmody, R.N. et al. 2014. Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 505(7484): 559–563.
61. Fava, F., Gitau, R., Griffin, B.A. et al. 2013. The type and quantity of dietary fat and carbohydrate alter faecal microbiome and short-chain fatty acid excretion in a metabolic syndrome "at-risk" population. *Int J Obes (Lond)* 37(2): 216–223.
62. De Filippo, C., Cavalieri, D., Di Paola, M. et al. 2010. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc Natl Acad Sci USA* 107(33): 14691–14696.
63. Schnorr, S.L., Candela, M., Rampelli, S. et al. 2014. Gut microbiome of the Hadza hunter-gatherers. *Nat Commun* 5: 3654. doi:10.1038/ncomms4654.
64. Fan, M.S., Zhao, F.J., Fairweather-Tait, S.J., Poulton, P.R., Dunham, S.J., and McGrath, S.P. 2008. Evidence of decreasing mineral density in wheat grain over the last 160 years. *J Trace Elem Med Biol* 22(4): 315–324, doi: 10.1016/j.jtemb.2008.07.002.
65. East, R. 2013. Microbiome: Soil science comes to life. *Nature* 501(7468): S18–S19. doi:10.1038/501S18a.
66. Hill, P.W., Marsden, K.A., and Jones, D.L. 2013. How significant to plant N nutrition is the direct consumption of soil microbes by roots? *New Phytol* 199(4): 948–955. doi:10.1111/nph.12320.

4 Clostridia Bacteria in the GI Tract Affecting Dopamine and Norepinephrine Metabolism

William Shaw, PhD

CONTENTS

INTRODUCTION

Ilya Ilyich Mechnikov, the Nobel Prize–winning biologist, was one of the first scientists to promote the idea that the microorganisms in the intestinal tract could affect health and was the first to recommend the use of beneficial lactobacilli to promote long life, essentially starting the probiotics (beneficial microorganisms) industry about a century ago. A Google search of the term *probiotics* yields 11,900,000 references. The term *dysbiosis* most commonly refers to an imbalance of microorganisms in the intestinal tract. A PubMed search indicates that the first appearance of this term in its database was in 1959 and that there are now 1005 uses of the term in articles in its database. However, the acceptance of this term took many years to gain general acceptance. Fifteen years ago, a bureaucrat from one of the laboratory regulatory agencies declared that this term was not permitted to be used by the laboratory of the author and that all such references would have to be deleted. For many years, it was thought by many in the medical community that the intestinal flora was an inert passenger and of little interest to health. Even a longer time passed until the role of intestinal microorganisms in brain function and psychiatric illness began to be studied in earnest. A PubMed

search of the terms "gut microbiota and psychiatric illness" retrieved 50 articles with the earliest published in 2008, while a search of "bacteria and psychiatric illness" retrieved 9244 articles dating back to 1947. Thus, infection was widely accepted as a potential cause of psychiatric disease and a worthy research subject, but the role of the intestinal flora in psychiatric disease was not readily accepted as a worthwhile research endeavor until recently.

DISCOVERY OF CLOSTRIDIA METABOLITES

At the children's hospital where the author was the laboratory director, he was given the project of setting up urine organic acids testing. At this time, urine organic acids testing was almost exclusively performed for diagnosis of inborn errors of metabolism. One of the first steps he took was to set up to test as many chemicals in urine as possible, not just those associated with genetic diseases. He tested for food additives, vitamins, minerals, a variety of drug metabolites, and other substances. He then approached the director of a children's psychiatric hospital to begin screening for metabolic causes of psychiatric diseases. The hospital director, a psychiatrist, indicated that he had the perfect patient for the project, a teenage boy with a variety of severe behavior disorders including depression, attention deficit disorder, and oppositional defiant disorder. He then said he was certain this boy had a biological cause of his illnesses, not bad parenting, and that he would send a urine sample. An analysis of his sample indicated that his patient had an increase of the chemical later identified as 3-(3-hydroxyphenyl)-3-hydroxypropionic acid (HPHPA) in the urine. The child's urine was tested while he was in outpatient treatment for attention deficit, oppositional defiant disorder, and depression, and it revealed high concentrations of the HPHPA compound (Figure 4.1). At a later date, the condition of the boy deteriorated, he was hospitalized, and another urine sample was submitted. This time the concentration of the HPHPA was massive, so massive that it obliterated nearly all of the other peaks in the chromatogram and could not be quantitated. The amount, however, was likely 1000 times or more than amounts found in normal individuals.

Studying metabolism in patients with mental illness is very challenging because many of the patients are on a variety of drugs, with each drug having multiple metabolites. Many researchers have wasted years identifying a new compound found in urine, only to discover that they had merely found a new drug metabolite. In a study by this author and Dr. Walter Gattaz, a research psychiatrist at the Central Mental Health Institute of Germany in Mannheim, drug-free samples from patients with schizophrenia diagnoses were evaluated. Five of the 12 samples (41.7%) contained a very high concentration of a compound identified by gas chromatography/mass spectrometry (GC/MS) as a derivative of the amino acid phenylalanine, which has since been identified as HPHPA.

Despite the seeming novelty of this compound's identification, it had been referenced 50 years prior.[1] This research indicated that HPHPA, which they called (-)-beta-meta-hydroxyphenylhydracrylic

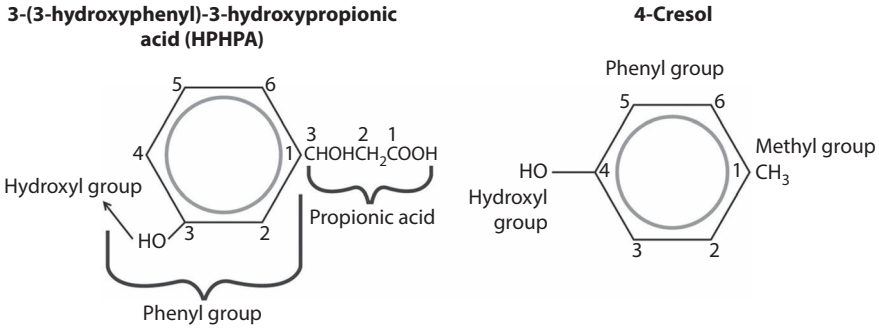

FIGURE 4.1 Biochemical structures of phenolic metabolites derived from clostridia bacteria.

acid, had been identified as one of the major phenolic acids present in elevated amounts in urine samples of patients with a large variety of mental illnesses. This association of elevated HPHPA in mental illness has been confirmed by the author. Remarkably, there was no follow-up of this important discovery by the earlier research team or any other research groups until the author's rediscovery. A similar discovery of genetic factors in peas (eventually recognized as the chromosomes) by the monk Mendel in 1865 was mostly overlooked for 35 years until rediscovered in 1900 by Hugo de Vries, Carl Correns, and Erich von Tschermak.[2]

Later, Shaw et al. found that HPHPA was elevated in many children with autism[3] and initially suspected that this compound might be an intestinal yeast metabolite. However, the author found that in a clinical research project on the effects of antifungal drugs on autism, there was not a significant decrease in HPHPA in the urine after antifungal drug therapy.[4] The mean value for the HPHPA actually increased some after antifungal therapy. This increase indicated to the author that this compound could not be due to the yeast but was probably due to a different microorganism. Since several children and adults with *Clostridium difficile* infection of the intestinal tract had high values of HPHPA in their urine, and the production of a similar compound, monohydroxyphenylpropionic acid is characteristic of different species of clostridia[5,6] but not other bacteria, I suspected that one or more species of the bacteria genus *Clostridium* were producing this compound.

KNOWN DISEASE-CAUSING CLOSTRIDIA BACTERIA

Some of the common species of *Clostridium* are *Clostridium tetani* that causes tetanus, *C. botulinum* that causes the food poisoning botulism, and *C. perfringens* and *C. difficile* that cause diarrhea.[7] *C. perfringens*, *C. novyi*, *C. bifermentans*, *C. histolyticum*, *C. septicum*, and *C. fallax* may all cause gangrene.[7] Many other species of *Clostridium* are normal inhabitants of the intestinal tract and may not be scientifically described or even named as a species. It is estimated that there are perhaps 100 different species of clostridia bacteria in the human intestine, but most are not considered pathogenic. The major reason for a lack of knowledge about these organisms is that they are strict anaerobes that cannot tolerate oxygen. Since they must be processed in an oxygen-free environment, most hospital laboratories do not have the capability to identify these organisms by culture methods.

The exception is *C. difficile*, which is most commonly identified by the toxins it produces in the stool rather than by the isolation of the organism. *C. difficile* overgrowth of the intestinal tract causes a severe and potentially fatal disorder called *pseudomembranous colitis*.[8] This overgrowth is frequently associated with the use of oral antibiotics, indicating that this organism is resistant to many of the common antibiotics such as penicillin, ampicillin, tetracyclines, cephalosporins, chloramphenicol, and others.[9] This organism is usually treated with either metronidazole (Flagyl®) or vancomycin followed by a replenishment of the intestine with *Lactobacillus acidophilus*.[8] Since many bacteria can genetically transfer drug resistance to other similar species and even unrelated species, it is my opinion that multiple species of clostridia may now be resistant to the most common antibiotic drugs.

4-CRESOL AS AN ADDITIONAL URINARY MARKER FOR *CLOSTRIDIUM DIFFICILE*

In addition to the discovery of HPHPA as a marker for gastrointestinal (GI) clostridial overgrowth, 4-cresol (para-cresol) (Figure 4.1) has been used as a specific marker for *C. difficile*.[10] 4-Cresol, a phenolic compound, is classified as a type-B toxic agent and can cause rapid circulatory collapse and death in humans.[11] Yokoyama et al.[12] recently proposed that intestinal production of 4-cresol may be responsible for a growth-depressing effect on weanling pigs. Signs of acute 4-cresol toxicity

in animals typically include hypoactivity, salivation, tremors, and convulsions. Clinical signs of toxicity[13] following inhalation include irritation of mucous membranes, excitation and convulsions, hematuria at high 4-cresol concentrations, and death. High amounts of 4-cresol have been found in autism,[13] and the amount of 4-cresol in the urine has been found elevated in initial samples and in replica samples of autistic children below 8 years of age. Increased levels of this compound in autism are correlated with the degree of severity of symptoms. 4-Cresol is apparently produced by *C. difficile* as an antimicrobial compound that kills other species of bacteria in the GI tract, allowing the *C. difficile* to proliferate.

MECHANISM BY WHICH CLOSTRIDIA METABOLITES ALTER HUMAN NEUROTRANSMITTER METABOLISM AND CAUSE PSYCHIATRIC DISORDERS AND GI DISEASE

The probable biochemical mechanism by which the phenolic compounds affect behavior was discovered by Goodhart et al.[14] who found that 4-cresol and similar phenols are potent inhibitors of dopamine-beta-hydroxylase, a key enzyme in catecholamine metabolism, which is responsible for converting dopamine to norepinephrine. 4-Cresol and related phenols are postulated to covalently modify dopamine-beta-hydroxylase by a direct insertion of an aberrant 4-cresol radical into an active site tyrosine residue at position 216 of the protein, leading to irreversible inactivation of the enzyme. It is interesting that the *inactivation* of the enzyme requires vitamin C (ascorbic acid), so vitamin C *deficiency* might actually offer protection against the negative effects of this enzyme inhibitor. This fact could be very clinically important, and vitamin C supplementation should probably be avoided until clostridia treatment has been completed. Although HPHPA has not yet been verified to be an inhibitor of dopamine-beta-hydroxylase, its structure is similar to 4-cresol (Figure 4.1), and many similar phenols have been proved to be strong inhibitors of this enzyme.[14] In addition, a number of observations of organic acid test results provide indirect evidence for dopamine-beta-hydroxylase inhibition by HPHPA.

The author observed that urine samples of many individuals with severe clinical symptoms of neuropsychiatric diseases had elevated levels of the dopamine metabolite homovanillic acid (HVA), while the epinephrine and norepinephrine metabolite vanillylmandelic acid (VMA) was not elevated or was low. At the same time, the ratio of the two metabolites or the HVA/VMA ratio was extremely elevated. The same urine samples for these individuals commonly had elevated amounts of the phenolic substances 4-cresol or HPHPA. Elevated HPHPA and/or 4-cresol have now been detected in a wide variety of disorders (Table 4.1).

TABLE 4.1
Disorders Associated with Elevated Amounts of Urinary Metabolites HPHPA and/or 4-Cresol from Clostridia Bacteria

Autism and Pervasive Developmental Disorder	Obsessive Compulsive Disorder
Attention deficit	Tic disorders
Arthritis	Tourette's syndrome
Depression	Parkinson's syndrome
Bipolar depression	Alzheimer's disease
Ulcerative colitis	Psychosis
Anorexia	Schizophrenia
Anxiety	Seizure disorder
Chronic fatigue syndrome	

METABOLIC PATHWAY FOR PRODUCTION OF HUMAN NEUROTRANSMITTERS INHIBITED BY CLOSTRIDIA METABOLITES

The combined metabolic pathway for production of human neurotransmitters in the brain, adrenal glands, and sympathetic nervous system is outlined in Figure 4.2 along with the production of clostridia bacterial substances that alter this pathway. The key starting material for both human and clostridia pathways is the amino acid phenylalanine. The human pathway requires the L-isomer. The specificity for the amino acid optical isomer required for the clostridia pathway is unknown.

In humans, phenylalanine and/or tyrosine from dietary proteins or amino acid supplements are absorbed from the intestinal tract where these amino acids cross the blood-brain barrier and enter the brain. Phenylalanine in the brain is converted to tyrosine by phenylalanine hydroxylase. The ring of tyrosine is then hydroxylated to dihydroxyphenylalanine (DOPA) by tyrosine hydroxylase. DOPA is then converted to dopamine by DOPA decarboxylase which requires a vitamin B_6 cofactor. The fate of further dopamine metabolism depends on the neuron type. In dopamine-containing neurons, dopamine is the final product. In the absence of clostridia metabolites, dopamine is converted primarily to homovanillic acid which can be measured in the urine organic acid test. In norepinephrine-containing brain neurons, neurons of the peripheral central nervous system, and

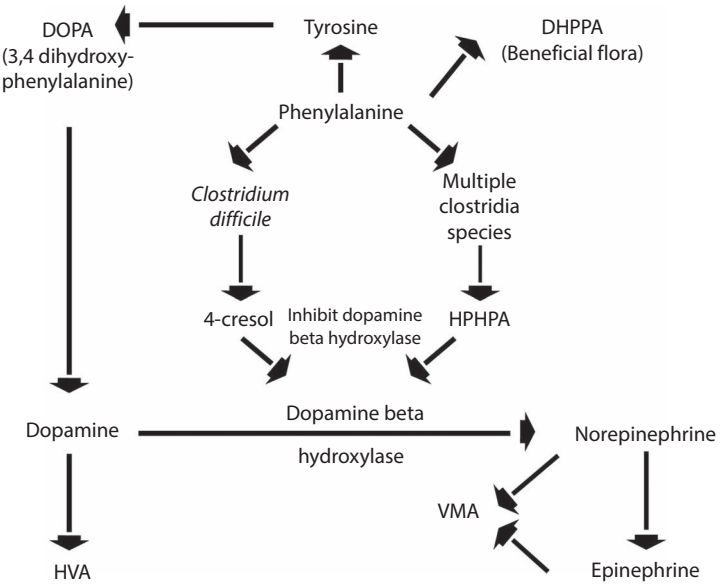

FIGURE 4.2 Integration of human and microbial metabolism involving neurotransmitters as cause of neuropsychiatric disorders. In the absence of 4-cresol and HPHPA from clostridia bacteria, phenylalanine in the central and peripheral nervous systems is converted in sequence to tyrosine, 3,4-dihydroxyphenylalanine (DOPA), dopamine, and norepinephrine. Norepinephrine may be converted to epinephrine in the adrenal gland. Dopamine is metabolized to homovanillic acid (HVA) while norepinephrine and epinephrine are converted to vanillylmandelic acid (VMA). When certain clostridia bacteria are present in the gastrointestinal tract, phenylalanine is increasingly converted to 4-cresol or 3-(3-hydroxyphenyl)-3-hydroxypropionic acid (HPHPA). This conversion depletes the supply of phenylalanine to produce neurotransmitters. In addition, 4-cresol and HPHPA inhibit the conversion of dopamine to norepinephrine by the irreversible inhibition of the key enzyme dopamine beta-hydroxylase. The inhibition of dopamine beta-hydroxylase leads to an imbalance of neurotransmitters in which dopamine is elevated and norepinephrine is depressed. Elevated dopamine can cause depletion of glutathione and increased oxidative species, leading to brain damage.