The Use of Mood Stabilizers as Plasticity Enhancers in the Treatment of Neuropsychiatric Disorders

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Mood disorders have traditionally been conceptualized as neurochemical disorders, but there is now evidence from a variety of sources demonstrating regional reductions in central nervous system (CNS) volume, as well as reductions in the numbers and/or sizes of glia and neurons in discrete brain areas. Although the precise cellular mechanisms underlying these morphometric changes remain to be fully elucidated, the data suggest that severe mood disorders are associated with impairments of structural plasticity and cellular resilience. It is thus noteworthy that lithium and valproate have recently been demonstrated to robustly increase the expression of the cytoprotective protein bcl-2 (an abbreviation for the B-cell lymphoma/leukemia-2 gene) in the CNS in vivo and in cells of human neuronal origin. Lithium and valproate also robustly activate a signaling cascade utilized by endogenous growth factors—the extracellular signal-regulated kinase (ERK) mitogen-activated protein (MAP) kinase pathway. Complementary human studies have shown that chronic lithium administration significantly increases gray matter content in a regionally selective manner, suggesting a reversal of illness-related atrophy and an increase in the volume of the neuropil. These unique and unexpected properties of lithium and valproate suggest that they may have broader utility as adjunctive agents in the treatment of a variety of neuropsychiatric disorders associated with cell atrophy or loss. The adjunctive use of these agents—at low doses—may provide the trophic support necessary to restore, enhance, and maintain normal synaptic connectivity, thereby allowing the chemical signal to reinstate the optimal functioning of critical circuits necessary for normal functioning.

(J Clin Psychiatry 2003;64[suppl 5]:3–17)

Lithium is an element discovered over 175 years ago (1817), and, over the ensuing period of time, it has been utilized in various formulations as a remedy for a multitude of maladies afflicting the human body.[1] However, it was not until its serendipitous rediscovery by the Australian physician/scientist John Cade 54 years ago, and subsequent clinical studies by Mogens Schou in the early 1950s, that lithium was seen by modern psychiatry as an effective antimanic treatment and a prophylactic therapy for manic-depressive illness (bipolar affective disorder, bipolar disorder [BD]).[2] The discovery of lithium's efficacy as a mood-stabilizing agent has since revolutionized the treatment of patients with BD—indeed, it is likely that the remarkable efficacy of lithium has served to spark a revolution that has, over time, reshaped not only medical and scientific but also popular concepts of severe mental illnesses. After nearly 3 decades of use in North America, lithium continues to be the mainstay of treatment for BD, both for the acute manic phase and as prophylaxis for recurrent manic and depressive episodes.[3] Adequate lithium treatment, particularly in the context of a lithium clinic, is also reported to reduce the excessive mortality observed in the illness,[4,5] and it has been estimated that in most industrialized countries, 0.1% of the population is undergoing lithium treatment.[6] Additionally, lithium is one of the most successful augmentations to antidepressants in treatment-resistant unipolar depression.[7] The effect on the broader community is highlighted by one estimation that the use of lithium saved the United States $4 billion in the period 1969–1979 by reducing associated medical costs and restoring productivity.[8] However, despite its role as one of psychiatry's most important treatments,[3] the biochemical basis for lithium's antimanic and mood-stabilizing actions remains to be fully elucidated.[9] Determination of the mechanism(s) by which lithium stabilizes an underlying

dysregulation of limbic and limbic-associated function also offers the potential to delineate the underlying etiology/ pathophysiology of BD.[9] Recognition of the significant morbidity and mortality of the severe mood disorders, as well as the growing appreciation that a significant percentage of patients respond poorly to existing treatments, has made the task of discovering new therapeutic agents that work quickly, potently, specifically, and with few side effects increasingly more important. Indeed, in a recent article discussing the major impact this monovalent cation has had on psychiatry, Schou expressed disappointment that we have yet to fully elucidate the mechanisms by which lithium exerts its therapeutic effects.[10]

A recognition of the clear need for better treatments and the lack of significant advances in our ability to develop novel, improved therapeutics for these devastating illnesses has led to the investigation of the putative roles of intracellular signaling cascades and non-aminergic systems in the pathophysiology and treatment of mood disorders. Consequently, recent evidence demonstrating that impairments of neuroplasticity and cellular resilience may underlie the pathophysiology of mood disorders, and that antidepressants and mood stabilizers exert major effects on signaling pathways that regulate neuroplasticity and cell survival, has generated considerable excitement among the clinical neuroscience community and is reshaping views about the neurobiological underpinnings of these disorders.[11]

"Neuroplasticity" subsumes diverse processes of vital importance by which the brain perceives, adapts to, and responds to a variety of internal and external stimuli. The manifestations of neuroplasticity in the adult central nervous system (CNS) have been characterized as including alterations of dendritic function, synaptic remodeling, long-term potentiation and depression, axonal sprouting, neurite extension, synaptogenesis, and even neurogenesis.[12] Although the potential relevance of neuroplastic events for the pathophysiology of psychiatric disorders has been articulated for some time, recent morphometric studies of the brain (both in vivo and postmortem) are beginning to lead to a fuller appreciation of the magnitude and nature of the neuroplastic events involved in the pathophysiology of mood disorders. In this article, we review these data and discuss their implications not only for changing existing conceptualizations regarding the pathophysiology of severe, recurrent mood disorders, but also for the strategic development of improved therapeutics.

EVIDENCE FOR IMPAIRMENTS OF STRUCTURAL PLASTICITY AND CELLULAR RESILIENCE IN MOOD DISORDERS

Positron emission tomography imaging studies have revealed multiple abnormalities of regional cerebral blood flow and glucose metabolism in limbic and prefrontal cortical (PFC) structures in mood disorders. These abnormalities implicate limbic-thalamic-cortical and limbic-cortical-striatal-pallidal-thalamic circuits, involving the amygdala, orbital and medial PFC, and anatomically related parts of the striatum and thalamus in the pathophysiology of mood disorders. Interestingly, recent morphometric magnetic resonance imaging (MRI) and postmortem investigations have also demonstrated abnormalities of brain structure that persist independently of mood state and may contribute to the corresponding abnormalities of metabolic activity (as discussed elsewhere).[13–16]

Structural imaging studies have demonstrated reduced gray matter volumes in areas of the orbital and medial PFC, ventral striatum, and hippocampus and enlargement of the third ventricle in mood disordered relative to healthy control samples, which has been reviewed.[17–19] Also consistent is the presence of white matter hyperintensities (WMH) in the brains of elderly depressed patients and patients with BD; these lesions may be associated with poor treatment response.[20] There is a growing awareness of the genetic influences on WMH, and their possible impact on neuropsychological functioning,[21] but WMH may have multiple causes, including cerebrovascular accidents, demyelination, loss of axons, dilated perivascular space, minute brain cysts, and necrosis.[20] In this context, it is noteworthy that recent studies have used diffusion tensor imaging of brain tissue to study possible white matter tract disruption in mood disorders.[22] This procedure measures diffusion of water as influenced by tissue structure, providing a measure of the integrity of fiber tracts. These authors showed that WMH showed a higher apparent diffusion coefficient and lower anisotropy than normal regions, with gray matter showing similar trends. Together, these results support the contention that WMH damage the structure of brain tissue and most likely disrupt the neuronal connectivity necessary for normal affective functioning. Although the cause of WMH in mood disorders is unknown, their presence—particularly in the brains of young bipolar patients—suggests importance in the pathophysiology of the disorder.[20,23]

Complementary postmortem neuropathologic studies have shown abnormal reductions in cortex volume, glial cell counts, and/or neuron size in the subgenual PFC, orbital cortex, dorsal anterolateral PFC, and amygdala (Table 1; recently reviewed[14,24,29]). It is not known whether these deficits constitute developmental abnormalities that may confer vulnerability to abnormal mood episodes, compensatory changes to other pathogenic processes, or the sequelae of recurrent affective episodes per se. Understanding these issues will partly depend on experiments that delineate the onset of such abnormalities within the illness course and determine whether they antedate depressive episodes in individuals at high familial risk for mood disorders. While there is not total reproducibility among either the neuroimaging or postmortem studies, the differences most likely represent variations of experimental design (including medication effects, vide infra) and, as would be expected in heteroge-

neous disorders such as mood disorders, in patient populations. Thus, research is required in order to understand if more rigorously defined subtypes of depression, or mood disorders, are associated with any particular abnormality.[20] Nevertheless, the marked reduction in glial cells in these regions has been particularly intriguing in view of the growing appreciation that glia play critical roles in regulating synaptic glutamate concentrations and CNS energy homeostasis and in releasing trophic factors that participate in the development and maintenance of synaptic networks formed by neuronal and glial processes.[30–33] Abnormalities of glial function could thus prove integral to the impairments of structural plasticity and overall pathophysiology of mood disorders.

BCL-2 AS A THERAPEUTICALLY RELEVANT TARGET FOR THE ACTIONS OF LITHIUM AND VALPROATE: THE SUCCESSFUL APPLICATION OF A CONCERTED mRNA DIFFERENTIAL DISPLAY STRATEGY TO IDENTIFY NOVEL TARGET GENES

It has become increasingly appreciated in recent years that the long-term treatment of mood disorders most likely involves the strategic regulation of signaling pathways and gene expression in critical neuronal circuits.[9,33–36] In this context, several independent laboratories have now demonstrated that lithium and valproate, at therapeutically relevant concentrations, produce complex alterations in basal and stimulated DNA binding to activator protein 1 (AP-1) transcription factors.[37–40] Together, these data suggest that lithium and valproate, via their effects on the AP-1 family of transcription factors, may bring about strategic changes in gene expression in critical neuronal circuits, effects that may ultimately underlie its efficacy in the treatment of a very complex neuropsychiatric disorder. Although many genes that are the targets of long-term lithium and valproate treatment have indeed been identified, it has been estimated that ≈ 10,000 to 15,000 genes may be expressed in a given cell at any time, and thus additional, novel methodologies are clearly required to study the complex pattern of gene expression changes induced by chronic drug treatment.

One methodology that has been successfully utilized to identify the differential expression of multiple genes (e.g., in pathologic vs. normal tissue or in control vs. treated tissue) is reverse transcription polymerase chain reaction mRNA differential display. Using this method, Wang and Young[35] were the first to make the novel observation that lithium increased 2',3'-cyclic nucleotide 3'-phosphodiesterase mRNA levels in C6 glioma cells. Subsequent studies have also utilized this methodology to identify novel genes that may be the targets for the actions of mood stabilizers. A major problem inherent in neuropharmacologic research, however, is the dearth of phenotypic changes clearly associated with treatment response, particularly for mood-stabilizing agents.[41] In the absence of clear-cut phenotypic

Table 1. Postmortem Morphometric Brain Studies in Mood Disorders Demonstrating Cellular Atrophy and/or Loss[a]

Reduced volume/cortical thickness

Cortical thickness rostral orbitofrontal cortex in MDD

Laminar cortical thickness in layers III, V, and VI in subgenual anterior cingulate cortex (area 24) in BD

Volume of subgenual prefrontal cortex in familial MDD and BD

Volumes of nucleus accumbens (left), basal ganglia (bilateral) in MDD and BD

Parahippocampal cortex size (right) in suicide

Reduced neuronal size and/or density

Pyramidal neuronal density, layers III and V in dorsolateral prefrontal cortex in BD and MDD

Neuronal size in layer V (–14%) and VI (–18%) in prefrontal cortex (area 9) in BD

Neuronal size in layer VI (–20%) in prefrontal cortex (area 9) in MDD

Neuronal density and size in layers II through IV in rostral orbitofrontal cortex, in layers V/VI in caudal orbitofrontal cortex, and in supragranular and infragranular layers in dorsolateral prefrontal cortex in MDD

Neuronal size in layer VI (–23%) in anterior cingulate cortex in MDD

Neuronal density in layers III, V, and VI in subgenual anterior cingulated cortex (area 24) in BD

Layer-specific interneurons in anterior cingulate cortex in BD and MDD

Non-pyramidal neuronal density in layer II (–27%) in anterior cingulate cortex in BD

Non-pyramidal neuronal density in the CA2 region in BD

Reduced glia

Density/size of glia in dorsolateral prefrontal cortex and caudal orbitofrontal cortex in MDD and BD—layer specific

Glial cell density in sublayer IIIc (–19%) (and a trend to decrease in layer Va) in dorsolateral prefrontal cortex (area 9) in BD

Glial number in subgenual prefrontal cortex in familial MDD (–24%) and BD (–41%)

Glial cell density in layer V (–30%) in prefrontal cortex (area 9) in MDD

Glial cell density in layer VI (–22%) in anterior cingulate cortex in MDD

Glial cell counts, glial density, and glia-to-neuron ratios in amygdala in MDD

[a]Modified and reproduced with permission from Manji and Duman,[13] with additional information from references 24–28.
Abbreviations: BD = bipolar disorder, MDD = major depressive disorder.

changes, we have attempted to overcome this experimental hurdle by utilizing paradigms that involve the identification of common long-term molecular targets of structurally dissimilar mood-stabilizing agents (lithium and valproate) when administered chronically in vivo.[42] These 2 distinct agents undoubtedly also exert a variety of dissimilar effects. However, the identification of genes affected by both agents, when administered in a therapeutically relevant paradigm, suggests that such molecular changes may play a role in the common therapeutic effects of these agents.

One of the genes whose expression was markedly increased by the treatments is the transcription factor polyoma enhancer binding protein (PEBP2β; GenBank Accession Number: AF087437).[42] It was further demonstrated that the function of PEBP2β (DNA binding of the PEBP2αβ complex) was also clearly increased in frontal cortex by both lithium and valproate, but not by chronic amphetamine or benzodiazepine.[42] One critical gene whose expression is known to be regulated by PEBP2β is the major neuroprotective protein bcl-2 (an abbreviation for the B-cell lymphoma/leukemia-2 gene); it was subsequently demonstrated that chronic treatment of rats with both

Figure 1. Effects of Chronic Lithium on the Immunolabeling of Bcl-2 in Rat Brain[a]
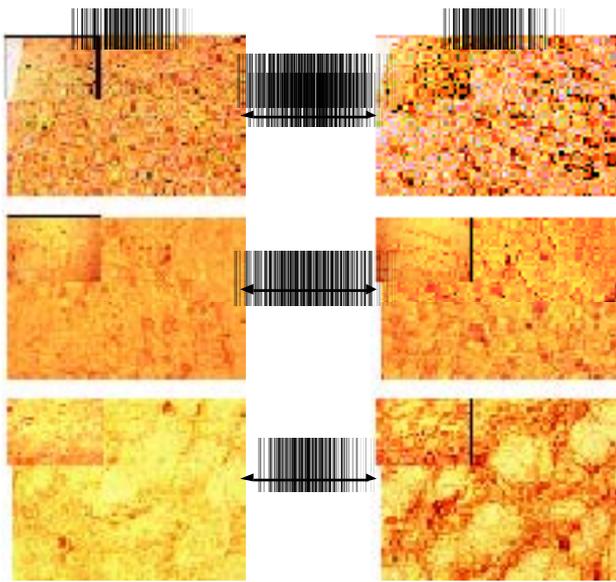
[a]Reprinted with permission from Manji et al.[44] Wistar Kyoto rats were treated with lithium carbonate for 4 weeks (twice-daily injection). Blood lithium levels were found to be in the therapeutic range (0.7 ± 0.3 mmol/L). Main photographs used 40× magnification; insets used 10× magnification.

agents resulted in a doubling of bcl-2 levels in frontal cortex. Furthermore, immunohistochemical studies showed that chronic treatment of rats with lithium or valproate resulted in a marked increase in the number of bcl-2 immunoreactive cells in layers II and III of frontal cortex.[42] Interestingly, the importance of neurons in layers II through IV of the frontal cortex in mood disorders has recently been emphasized, since primate studies have indicated that these are important sites for connections with other cortical regions and major targets for subcortical input.[32] Chronic lithium also markedly increased the number of bcl-2 immunoreactive cells in the dentate gyrus and striatum[43] (Figure 1); detailed immunohistochemical studies following chronic valproate treatment are currently underway. We have subsequently demonstrated that lithium also increases bcl-2 levels in C57BL/6 mice[45] and in human neuroblastoma SH-SY5Y cells in vitro[43]; our demonstration of a lithium-induced increase in bcl-2 levels has also been convincingly replicated in rat cerebellar granule cells in a recent study.[46] The latter study was undertaken to investigate the molecular and cellular mechanisms underlying the neuroprotective actions of lithium against glutamate excitotoxicity (vide infra). These investigators found that lithium produced a remarkable increase in bcl-2 protein and mRNA levels. Moreover, lithium has recently been demonstrated to *reduce* the levels of pro-apoptotic protein (p53) both in cerebellar granule cells[46] and SH-SY5Y cells.[47] Thus, overall, the data clearly show that chronic lithium robustly increases the lev-

els of the neuroprotective protein bcl-2 in areas of rodent frontal cortex, hippocampus, and striatum in vivo and in cultured cells of both rodent and *human neuronal origin* in vitro; furthermore, at least in cultured cell systems, lithium has also been demonstrated to reduce the levels of p53.

Bcl-2 was the first identified member of a large family of cellular and viral apoptosis-regulating proteins.[48–50] The bcl-2 family is the best-characterized protein family involved in the regulation of apoptotic cell death and consists of both anti-apoptotic (e.g., bcl-2 and bcl-XL) and pro-apoptotic members (e.g., BAX and BAD), several of which are expressed in the rodent and mammalian CNS. Bcl-2 attenuates apoptosis by sequestering proforms of death-driving cysteine proteases (called "caspases"); by preventing the release of mitochondrial apoptogenic factors such as calcium, cytochrome c, and apoptosis-inducing factor into the cytoplasm; and by enhancing mitochondrial calcium uptake (recently reviewed[49,50]). Increasing evidence suggests a critical role for mitochondria in the process of apoptosis, and studies have shown that mitochondria undergo major changes in membrane integrity before classical signs of apoptosis become manifest, leading to a disruption of the inner transmembrane potential ($\Delta\psi_m$) and the release of intermembrane proteins through the outer membrane.[51] Chemically induced opening or closing of the permeability transition pore (also called "mitochondrial megachannel") can induce or prevent apoptosis, respectively. Bcl-2 acts on mitochondria to stabilize membrane integrity and to prevent opening of the permeability transition pore, and has been shown to protect neurons from a variety of insults both in vitro and in vivo.[49,50] Overexpression of bcl-2 in transgenic mice has been shown to prevent motor neuron and retinal ganglion death, to protect cells from the deleterious effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or focal ischemia, to protect photoreceptor cells from inherited retinal degeneration, and to prolong survival and attenuate motor neuron degeneration in a transgenic animal model of amyotrophic lateral sclerosis.[44,48–50]

Accumulating data suggest that not only is bcl-2 neuroprotective, but it also exerts neurotrophic effects and promotes neurite sprouting, neurite outgrowth, and axonal regeneration.[52–54] Moreover, a recent study demonstrated that severe stress exacerbates stroke outcome by suppressing bcl-2 expression.[55] In this study, the stressed mice expressed ≈ 70% less bcl-2 mRNA than unstressed mice after ischemia. Furthermore, stress greatly exacerbated infarct in control mice but not in transgenic mice that constitutively express increased neuronal bcl-2. Finally, high corticosterone concentrations were significantly correlated with larger infarcts in wild-type mice, but not in bcl-2 overexpressing transgenic mice. Thus, enhanced bcl-2 expression appears to be capable of offsetting the potentially deleterious consequences of stress-induced neuronal endangerment and suggests that pharmacologically induced up-regulation of

bcl-2 may have considerable utility in the treatment of a variety of disorders associated with endogenous or acquired impairments of cellular resilience (vide infra). Overall, it is clear that the neurotrophic factor/mitogen-activated protein (MAP) kinase/bcl-2 signaling cascade plays a critical role in cell survival in the CNS and that there is a fine balance maintained between the levels and activities of cell survival and cell death factors; modest changes in this signaling cascade or in the levels of the bcl-2 family of proteins (potentially due to genetic-, illness-, or insult-related factors) may therefore profoundly affect cellular viability.

THE ROLE OF GENE EXPRESSION PROFILING IN PSYCHIATRIC RESEARCH

As described above, differential display (DD) technology was used to discover the up-regulation of bcl-2 by lithium and valproate (via the identification of the up-regulation of a transcription factor known to regulate bcl-2—PEBP2β). The discovery of this unexpected target, which has had a major influence on our thinking about the long-term cellular effects of mood stabilizers, illustrates the importance of hypothesis-generating (as opposed to hypothesis-dependent) techniques. Microarrays are another example of this type of research tool and have already proved quite valuable in the study of psychiatric drugs. As the technology improves, current problems are eliminated, and new applications are developed, microarrays are likely to become an essential, indispensable tool for the neuroscience-psychiatric community. The methodological issues of microarray experiments have been extensively reviewed,[56–58] but are discussed here briefly.

It is important to note that microarray technology should not necessarily be considered to be superior to, or a replacement of, DD. Rather, each technique has unique advantages and disadvantages. Perhaps most obvious is the identification of transcripts: using a cDNA microarray, one is relatively certain what each differentially regulated mRNA is; with DD, on the other hand, one must clone and identify the transcript in question. While both methodologies require further validation to eliminate false-positives, the immediate identification of the differentially expressed transcripts by the microarray methodology translates into a major savings in terms of time and labor. However, this very advantage of the microarray technology is also one of its weaknesses compared with the DD methodology; the microarray methodology allows for the identification of known transcripts only (which the array in question is composed of); by contrast, DD allows for the detection of entirely novel mRNA transcripts and is thus truly unbiased. Oligonucleotide arrays are more like DD, in that novel targets may be identified and the identity of the transcript binding to a given oligo must be determined. For further comparison of DD and microarray technologies, the reader is referred to Liang's recent review.[59]

One key difficulty in the use of microarrays lies in the signal-to-noise ratio. Particularly for transcripts of low abundance, the test-retest reliability of microarray results can be dismal. For this reason, repetition is of obvious importance; noise can be filtered, and variance can be used to assess the validity of results.[60] Unfortunately, the high cost of microarrays often forces researchers to forgo replication entirely and thereby jeopardize the usefulness of their results. Likewise, the commonly used approach of establishing a threshold of the magnitude of expression ratio that constitutes a significant change fails to address the issue of deviation and error. As the reliability increases, and the cost decreases, microarray data will hopefully become subject to more statistically rigorous analyses.

Even given the importance of replication, the usefulness of conventional statistical measures, such as the t test, is another concern. In a set of 10,000 transcripts, it is clear that one can expect ≈ 500 false-positives, if one uses a $p < .05$ cutoff. On the other hand, if one uses Bonferroni correction for repeated analyses, one is all but guaranteed to find *no* significantly regulated mRNA, and thus to acquire numerous false-negatives.

In this light, the stringency with which microarray data are analyzed becomes a much fuzzier matter and may be adjusted to address the specific question under investigation. While at first glance one may question the scientific rigor of such an approach, it needs to be emphasized that the microarray methodology is only a screening technique, and the results require much more independent validation. Thus, the cutoff threshold criteria should be determined by not only statistical considerations, but also the stringency and rigor of the experimental paradigm (e.g., identifying common targets of structurally highly dissimilar drugs) and the ability/willingness to subsequently validate positive results with independent methodologies. For example, if an experimenter hopes to discover single genes that are the target of the manipulation, high stringency is probably warranted (e.g., $p < .01$ and effect size of 2-fold). This ensures that less time will be wasted on false-negatives at the polymerase chain reaction and/or protein validation stage. If, on the other hand, the goal is to find groups of related genes that are affected, or common targets of multiple agents, lower stringency may be more desirable. For example, if all 12 subtypes of a given receptor are up-regulated 30% with p values $\approx .10$, it may represent a very important finding that would have been discarded with higher stringency.

Aside from the t test and fold-difference comparisons, more powerful means of analyzing microarray data are emerging. Clustering is a fairly common technique in which transcripts are associated based on their co-regulation across a number of samples. "Fingerprinting" refers to the association of a general pattern of microarray results with a particular variable. For example, this technique has already begun to show usefulness in oncology, where patterns in microarray data from biopsies may help predict treatment

response and outcome.[61] Likewise, ex vivo gene expression data from psychiatric patients may one day predict optimal treatment strategies, risk of relapse, or other important clinical variables.

The Use of Microarrays to Ascertain the Actions of Mood Stabilizers

To maximize the meaningfulness of our microarray data, we have included a number of important design features in our experiments. As a first step in this important series of studies, we have utilized human neuroblastoma SH-SY5Y cells. These cells were selected for a number of reasons, including the following:

1. They are human cells with a neuronal phenotype.
2. They provide a very homogeneous population of cells and the ability to control most experimental conditions—critical to accurately identifying the test/retest stability of results, stability over time, and inter- and intra-assay coefficients of variations for the microarray methodology.
3. They have previously been shown to produce treatment-induced changes that are very similar to those that have been observed in critical areas of rodent brain during chronic in vivo treatment with mood stabilizers, including changes in protein kinase C isozymes, the extracellular signal-regulated kinase (ERK) MAP kinase cascade, the glycogen synthase kinase (GSK-3)/β-catenin pathway, bcl-2, and AP-1 DNA binding activity.[40,62–64]

For SY5Y experiments, 4 independent replicate control/experimental pairs were used for both lithium and valproate. Cells were treated for 5 days prior to mRNA extraction. For each pair, 2 slides were hybridized, reversing the Cy3/Cy5 labeling. This procedure corrects for the oft-encountered problem of gene-label interaction.[65] To model the requirement for chronic, systemic administration, rats were given lithium- or valproate-containing chow for 4 weeks, and hippocampi were collected bilaterally for mRNA extraction. Only animals with plasma drug levels within the therapeutic range were used for microarray analysis (lithium: 0.5–1.2 mM, valproate: 0.3–0.9 mM). Again, mRNA from each pair was hybridized to 2 slides, reversing the labels to control for gene-label interaction. Rat and SY5Y slides were read using the ScanArray 5000 (PerkinElmer Life Sciences, Boston, Mass.), and data were filtered and analyzed using QuantArray (PerkinElmer Life Sciences, Boston, Mass.) and Microsoft Excel (Microsoft, Redmond, Wash.). Replicates were tested for significance using a 2-tailed paired-samples t test.

The investment our laboratory has put into this intensive strategy has been rewarded. While specific reports are in progress and will appear elsewhere, some general trends are notable. A constellation of transcripts involved in apoptotic signaling have been found, in agreement with the large body of data supporting the cytoprotective effects of lithium and valproate. Therefore, it is a tempting hypothesis that mood stabilizers are inducing a cellular resilience program, which includes the host of alterations observed.

Interestingly, a number of genes involved in cytoskeletal functions were also dysregulated by lithium and valproate in SY5Y cells. Given the documented effects of lithium and valproate on processes like neurite outgrowth[62] and synaptic plasticity,[66–68] these results come as no surprise. Again, it is conceivable that the array of changes seen in cytoskeletal functions and gene expression represent the activation/suppression of cellular programs.

LITHIUM AND VALPROATE ROBUSTLY ACTIVATE A SIGNALING CASCADE UTILIZED BY ENDOGENOUS GROWTH FACTORS: THE ERK MAP KINASE CASCADE

Neurotrophic Signaling Cascades: A Focus on Brain-Derived Neurotrophic Factor

Neurotrophins are a family of regulatory factors that mediate the differentiation and survival of neurons, as well as the modulation of synaptic transmission and synaptic plasticity. The neurotrophin family now includes, among others, nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT3), NT4/5, and NT6. These various proteins are closely related in terms of sequence homology and receptor specificity. They bind to and activate specific receptor tyrosine kinases belonging to the Trk family of receptors, including TrkA, TrkB, TrkC, and a pan-neurotrophin receptor, p75.[69] Additionally, there are 2 isoforms of TrkB receptors: the full-length TrkB and the truncated form of TrkB, which does not contain the intracellular tyrosine kinase domain.[70] The truncated form of TrkB can thus function as a dominant-negative inhibitor for the TrkB receptor tyrosine kinase, thereby providing another mechanism to regulate BDNF signaling in the CNS.[71] Neurotrophins can be secreted constitutively or transiently, and often in an activity-dependent manner. Recent observations support a model wherein neurotrophins are secreted from the dendrite and act retrogradely at presynaptic terminals where they act to induce long-lasting modifications.[69] Within the neurotrophin family, BDNF is a potent physiologic survival factor that has also been implicated in a variety of pathophysiologic conditions. The cellular actions of BDNF are mediated through 2 types of receptors: a high-affinity tyrosine receptor kinase (TrkB) and a low-affinity pan-neurotrophin receptor (p75). TrkB is preferentially activated by BDNF and NT4/5 and appears to mediate most of the cellular responses to these neurotrophins.

BDNF and other neurotrophic factors are necessary for the survival and function of neurons,[72] implying that a sustained reduction of these factors could affect neuronal viability. However, what is sometimes less well appreciated is the fact that BDNF also has a number of much more acute

effects on synaptic plasticity and neurotransmitter release and facilitates the release of glutamate, γ-aminobutyric acid (GABA), dopamine, and serotonin.[73,74] In this context, BDNF has been shown to potentiate both excitatory and inhibitory transmission, albeit via different mechanisms; BDNF strengthens excitation primarily by augmenting the amplitude of α-amino-3-hydroxy-5-methylisoxazole-4-proprionic acid (AMPA) receptor–mediated miniature excitatory postsynaptic current, but enhances inhibition by increasing the frequency of miniature inhibitory postsynaptic current and increasing the size of GABAergic synaptic terminals. Furthermore, full-length TrkB receptor immunoreactivity has been found not only in glutamatergic pyramidal and granule cells, but also in some interneuron axon initial segments, axon terminals forming inhibitory-type synapses onto somata and dendritic shafts, and excitatory-type terminals likely to originate extrahippocampally. Together, these results suggest that TrkB is contained in some GABAergic interneurons, neuromodulatory (e.g., cholinergic, dopaminergic, and noradrenergic) afferents, and/or glutamatergic afferents.[75]

As discussed already, BDNF is best known for its long-term neurotrophic and neuroprotective effects, which may be very important for its putative role in the pathophysiology and treatment of mood disorders (vide infra). In this context, it is noteworthy that although endogenous neurotrophic factors have traditionally been viewed as increasing cell survival by providing necessary trophic support, it is now clear that their survival-promoting effects are mediated in large part by an inhibition of cell death cascades.[76] It is believed that neurotrophic factors inhibit cell death cascades by activating the MAP kinase signaling pathway and the phosphatidylinositol (PI) 3-kinase/Akt pathway. One important mechanism by which the MAP kinase signaling cascade inhibits cell death is by increasing the expression of the anti-apoptotic protein bcl-2.[77]

Lithium and Valproate Activate the ERK Signaling Cascade

In view of the important role of the ERK signaling cascade in mediating long-term neuroplastic events, a series of studies have been undertaken to investigate the effects of lithium and valproate on this signaling cascade.[62,78] These studies have shown that lithium and valproate, at therapeutically relevant concentrations, robustly activate the ERK MAP kinase cascade in human neuroblastoma SH-SY5Y cells.[62,78] Follow-up studies have recently shown that, similar to the effects observed in neuroblastoma cells in vitro, chronic lithium or valproate also robustly increases the levels of activated ERK in areas of brain that have been implicated in the pathophysiology and treatment of BD—the frontal cortex and hippocampus.[78] In addition to finding that valproate and lithium activate ERK, we have also found that downstream targets of the ERK pathway such as p90 ribosomal S6 kinase (RSK) and cyclic adenosine monophos-

phate response element binding protein (CREB) are activated, while the pro-apoptotic protein BAD is deactivated. Furthermore, an inhibitor of the enzyme that phosphorylates ERK, MAP kinase/ERK, attenuates the valproate-induced increase in ERK and RSK1 phosphorylation (G. Chen, M.D.; P. Yuan, M.D., Ph.D.; H.K.M.; unpublished observations, June 2002). Thus, recent evidence suggests that both lithium and valproate activate the ERK MAP kinase pathway and the transcription of downstream molecules bcl-2 and BDNF.

DOES INHIBITION OF GLYCOGEN SYNTHASE KINASE 3β PLAY A ROLE IN THE THERAPEUTIC EFFECTS OF LITHIUM?

While older literature suggests that lithium interacts with glycogen synthase,[79] it was not until 1996, when Klein and Melton[80] made the seminal observation that lithium inhibited the action of GSK-3, that the direct inhibition of this enzyme by lithium was identified. GSK-3 is a highly conserved enzyme in evolution and is found in 2 nearly identical isoforms in mammals, α and β.[81] This enzyme was first discovered (and named) on the basis of its ability to phosphorylate, and thereby inactivate, the enzyme glycogen synthase, an action that led to a decrease in the synthesis of glycogen. However, as we discuss below, GSK-3 is now known to regulate diverse functions in the adult mammalian brain. GSK-3 is unique among kinases in that it is constitutively active. Thus, most intracellular signals to GSK-3 inactivate the enzyme. Signals deactivating GSK-3 arise from insulin stimulation, numerous growth factors (for example, PI 3-kinase), and developmental signals. A number of endogenous growth factors (e.g., NGF and BDNF) utilize the PI 3-kinase signaling cascade as a major effector system. Thus, growth factors may bring about many of their neurotrophic/neuroprotective effects, at least in part, by GSK-3 inhibition. GSK-3 phosphorylates—and thereby inactivates—many targets, including transcription factors and cytoskeletal proteins such as the Alzheimer's protein *tau* (a previous name for GSK-3 was *tau* kinase). Inhibition of GSK-3 thus results in the release of this inhibition and activation of multiple cellular targets.

Lithium's inhibition of GSK-3 appears to be by competition with magnesium for a binding site.[82] Thus, magnesium binding increases the activity of GSK-3 when it is not inhibited by lithium.[82] As mentioned, in 1996, Klein and Melton first identified lithium as a direct inhibitor of GSK-3 activity and suggested that this finding may be relevant to lithium's efficacy in treating BD.[20,80,83] Three years later, valproic acid, another mood stabilizer commonly used in clinical practice, was also identified as an inhibitor of GSK-3, further suggesting the importance of this enzyme in BD research.[63,84] While lithium inhibits GSK-3 by direct competition with magnesium,[82] the precise mechanisms by which valproic acid exerts its action are still uncertain.[20]

Table 2. Neurotrophic and Neuroprotective Effects of Lithium[a]

Protects cultured cells of rodent and human neuronal origin in vitro from
  Glutamate
  High concentrations of calcium
  MPP$^+$
  β-Amyloid
  Aging-induced cell death
  HIV regulatory protein, Tat
  HIV gp120 envelope protein
  Glucose deprivation
  Growth factor or serum deprivation
  Toxic levels of anticonvulsants
  Platelet activating factor
  Aluminum toxicity
  Low potassium concentrations
  C2-ceramide
  Ouabain
  GSK-3β and staurosporine/heat shock
  β-Bungarotoxin
Enhances hippocampal neurogenesis in adult mice
Protects rodent brain in vivo from
  Cholinergic lesions
  Radiation injury
  Middle cerebral artery occlusion (model of stroke)
  HIV gp120 envelope protein injection (model of HIV-associated dementia)
  Quinolinic acid infusion (model of Huntington's disease)
  Aluminum maltolate
Human effects
  No subgenual PFC gray matter volume reductions in cross-sectional
    MRI studies
  No reductions in amygdala glial density in postmortem cell
    counting studies
  Increased total gray matter volumes on MRI compared with untreated
    BD patients in cross-sectional studies
  Increased NAA (marker of neuronal viability) levels in BD patients in
    longitudinal studies
  Increased gray matter volumes in BD patients in longitudinal studies

[a]Adapted from Manji et al.[44] (See also recent reviews by Li et al.[85] and Chuang et al.[108])
Abbreviations: BD = bipolar disorder, GSK-3β = glycogen synthase kinase 3β, HIV = human immunodeficiency virus, MPP$^+$ = 1-methyl-4-phenylpyridinium ion, MRI = magnetic resonance imaging, NAA = N-acetyl-aspartate, PFC = prefrontal cortex.

lesion, had small but significant effects on the recovery of cortical ChAT and AChE activity. Intriguingly, when applied in combination, the drugs clearly showed synergistic effects. However, considerable caution is required in the extrapolation of these results to the treatment of humans, since the coadministration of lithium and cholinesterase inhibitors has been shown to be capable of inducing seizures. In another study investigating lithium's effects against excitotoxic insults, it was demonstrated that lithium attenuated the kainic acid–induced reduction in glutamate decarboxylase levels and [^{3}H]D-aspartate uptake.[111] Chronic lithium has also been shown to exert dramatic protective effects against middle cerebral artery occlusion, reducing not only the infarct size (56%), but also the neurologic deficits (abnormal posture and hemiplegia).[112] Most recently, the same research group has demonstrated that chronic in vivo lithium treatment robustly protects neurons in the striatum from quinolinic acid–induced toxicity, in a putative model of Huntington's disease.[113] Table 2 summarizes some of the most robust experimental evidence demonstrating

neuroprotective effects of lithium in vitro and in vivo. In addition to lithium's effects on bcl-2 and GSK-3β, its effects on other signaling pathways and transcription factors[9,114] may also contribute to its neuroprotective effects. In this context, it is noteworthy that recent studies have shown that modulation of Akt-1 activity is involved in glutamate excitotoxicity and may play a role in lithium's neuroprotective effects in rat cerebellar granule cells.[115] Furthermore, Datta and colleagues[116] have demonstrated that Akt phosphorylation of BAD (a pro-apoptotic member of the bcl-2 family) blocks BAD-induced death of primary neurons. These results suggest that lithium's effects on Akt-1 may also contribute to neuroprotective effects; however, such a contention awaits the demonstration of lithium-induced activation of Akt-1 in the CNS in vivo.

Does Lithium Affect Neurogenesis?

As discussed already, utilizing a method for labeling cell division directly in the adult human brain, it has been shown that the dentate gyrus (an area where robust lithium-induced increases in bcl-2 levels are observed) can produce new neurons during adulthood in humans. A large number of the newborn daughter cells are known to die rapidly, most likely via apoptosis.[117] Thus, increasing bcl-2 levels could enhance the survival of the newborn cells, allowing them to differentiate into neurons. Additionally, bcl-2 has been shown to have robust effects on the *regeneration* of CNS axons.[118] In view of bcl-2's major neuroprotective and neurotrophic role, a study[45] was undertaken to determine if lithium, administered at therapeutically relevant concentrations, affects neurogenesis in the adult rodent brain. Kempermann and Gage[117] have suggested the use of the word *neurogenesis* to refer to a series of events (including proliferation of a neuronal precursor or stem cell, and survival of the daughter cells) that results in the appearance of a new neuron. To investigate the effects of chronic lithium on neurogenesis, mice were treated with "therapeutic" lithium (mean ± SD plasma levels = 0.97 ± 0.20 mM), for ≈ 4 weeks. After treatment with lithium for 14 days, the mice were administered single doses of bromodeoxyuridine (BrdU, a thymidine analog that is incorporated into the DNA of dividing cells) for 12 consecutive days. Lithium treatment continued throughout the duration of the BrdU administration. Following BrdU immunohistochemistry, 3-D cell counting was performed using a computer-assisted image analysis system. This system is based on the optical disector method and estimates the number of cells independent of section thickness and cell shape. We found that chronic lithium administration does, indeed, result in an increase in the number of BrdU-positive cells in the dentate gyrus[45] (Figure 2). Moreover, approximately two thirds of the BrdU-positive cells also double-stained with the neuronal marker NeuN, confirming their neuronal identity. Double-staining of BrdU and bcl-2 was also seen, and studies using bcl-2 transgenic animals are currently underway

Figure 2. Chronic Lithium Increases Hippocampal Neurogenesis[a]

A. BrdU Immunohistochemistry

B. Quantitation of BrdU-Positive Cells

C. Double-Labeling With BrdU and NeuN[b]
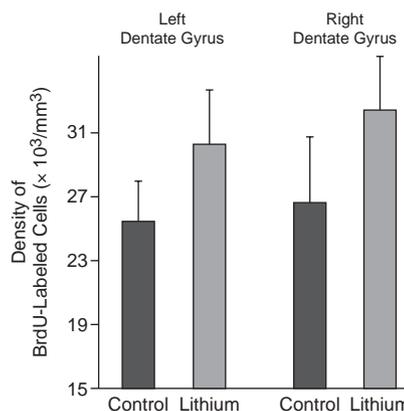
[a]Modified and reproduced with permission from Chen et al.[45] C57BL/6 mice were treated with lithium for 14 days and then received once-daily bromodeoxyuridine (BrdU) injections for 12 consecutive days while lithium treatment continued. Twenty-four hours after the last injection, the brains were processed for BrdU immunohistochemistry. Cell counts were performed in the hippocampal dentate gyrus at 3 levels along the dorsoventral axis in all the animals. BrdU-positive cells were counted using unbiased stereological methods. Chronic lithium produced a significant 25% increase in BrdU immunolabeling in both right and left dentate gyrus (p < .05).
[b]Neuron-specific nuclear protein, a neuronal marker.

to delineate the role of the observed bcl-2 overexpression in the enhanced hippocampal neurogenesis.

HUMAN EVIDENCE FOR THE NEUROTROPHIC EFFECTS OF LITHIUM

While the body of preclinical data demonstrating neurotrophic and neuroprotective effects of mood stabilizers is striking, considerable caution must clearly be exercised in extrapolating to the clinical situation with humans. In view of lithium's robust effects on the levels of the cytoprotective protein bcl-2 in the frontal cortex, Drevets[119] reanalyzed older data that demonstrated ≈ 40% reductions in subgenual PFC volumes in familial mood disorder subjects. Consistent with neurotrophic/neuroprotective effects of lithium, the results revealed that the patients treated with chronic lithium or valproate exhibited subgenual PFC volumes that were significantly higher than the volumes in patients not treated with lithium or valproate and not significantly different from control. In a more recent study, Bowley and colleagues[25] have investigated glial cell densities in mood disorder patients. Although the sample sizes are quite small, the authors made the intriguing observation that unipolar patients exhibited reduced glial cell densities, whereas only the bipolar patients off chronic lithium or valproate treatment exhibited similar reductions.

Although the results of the aforementioned studies suggest that mood stabilizers may have provided neuroprotective effects during naturalistic use, considerable caution is warranted in view of the small sample size and cross-sectional nature of the studies. To investigate the potential neurotrophic effects of lithium in humans more definitively,

a longitudinal clinical study was recently undertaken using proton magnetic resonance spectroscopy (MRS) to quantitate N-acetyl-aspartate (NAA, a putative marker of neuronal viability) levels.[120] Four weeks of lithium treatment produced a significant increase in NAA levels, effects that were localized almost exclusively to gray matter.[121] These findings provide intriguing indirect support for the contention that chronic lithium increases neuronal viability/function in the human brain. Furthermore, a ≈ 0.97 correlation between lithium-induced NAA increases and regional voxel gray matter content was observed, thereby providing evidence for co-localization with the regional specific bcl-2 increases observed (e.g., gray vs. white matter) in the rodent brain cortices. These results suggest that chronic lithium may not only exert robust neuroprotective effects (as has been demonstrated in a variety of preclinical paradigms), but also exert neurotrophic effects in humans.

Lithium Increases Gray Matter in the Human Brain

A follow-up volumetric MRI study has demonstrated that 4 weeks of lithium treatment also significantly increased total gray matter content in the human brain,[122] suggesting an increase in the volume of the neuropil (the moss-like layer composed of axonal and dendritic fibers that occupies much of the cortex gray matter volume; Figure 3). A finer-grained subregional analysis of these brain-imaging data is ongoing and clearly shows that lithium produces a regionally selective increase in gray matter, with prominent effects being observed in hippocampus and caudate.[123] Furthermore, no changes in overall gray matter volume are observed in healthy volunteers treated chronically with lithium, suggesting that lithium is truly producing a reversal of

Figure 3. Brain Gray Matter Volume Is Increased Following 4 Weeks of Lithium Administration at Therapeutic Levels in Bipolar Disorder Patients[a]

A. Gray Matter Segmentation

B.

C.
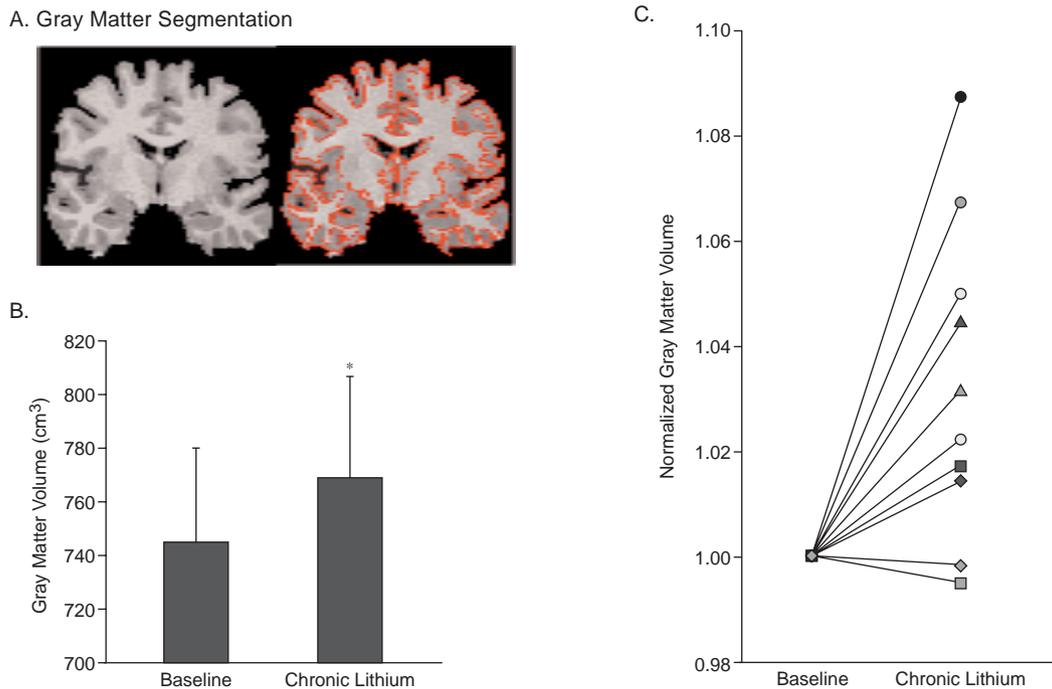
[a]Modified and reproduced with permission from Moore et al.[122] Inset (A) illustrates a slice of the 3-dimensional volumetric magnetic resonance imaging (MRI) data, which were segmented by tissue type using quantitative methodology to determine tissue volumes at each scan timepoint. (B) Brain tissue volumes were measured using high-resolution 3-dimensional MRI (124 images, 1.5-mm thick Coronal T1 weighted spoiled gradient echo images), and validated quantitative brain tissue segmentation methodology was used to identify and quantify the various components by volume, including total brain white and gray matter content. Measurements were made at baseline (medication free, after a minimum 14-day washout) and then repeated after 4 weeks of lithium at therapeutic doses. Chronic lithium significantly increases total gray matter content in the brain of patients with bipolar disorder. No significant changes were observed in brain white matter volume or in quantitative measures of regional cerebral water. Each symbol in inset (C) represents 1 patient.

illness-related atrophy, rather than nonspecific gray matter increases.

ARE TRADITIONAL "ANTIMANIC LEVELS" OF LITHIUM REQUIRED FOR NEUROTROPHIC/NEUROPROTECTIVE EFFECTS?

It is striking that lithium has such robust effects on the cytoprotective protein bcl-2, exerts neuroprotective effects in a variety of paradigms, and actually increases gray matter volumes in humans. These exciting results suggest that lithium may have utility in the treatment of a variety of neuropsychiatric disorders that are associated with cell atrophy/loss and impairments of cellular resilience. One obvious concern is lithium's tolerability, especially in patients with neurodegenerative disorders. Our laboratory has therefore undertaken a series of studies to determine if the chronic administration of lithium at low doses also regulates bcl-2 expression. We have found that chronic (4-week) lithium administration, at doses that produce plasma drug levels of ≈ 0.35 mM, robustly increases bcl-2 levels in rat frontal cortex (Figure 4) and hippocampus.[43] Furthermore, there is accumulating evidence from other groups suggesting that lithium exerts neuroprotective effects at lower doses. Thus, 0.5

mM of lithium has been shown to protect cultured cerebellar granule cells from glutamate excitotoxicity[124] and to decrease levels of the pro-apoptotic protein p53.[46] In middle cerebral artery occlusion, an in vivo model of stroke, lithium has also been shown to offer significant protection at 0.5 mEq/kg.[125] Of particular interest, a recent study demonstrated that cortical neurons are even more potently protected from excitotoxicity, with significant increases in viability occurring with lithium doses as low as 0.1 mM.[126] Overall, the data clearly suggest that doses of lithium lower than "traditional antimanic" doses have neurotrophic and neuroprotective effects and may thus have utility as adjunctive treatments for neuropsychiatric disorders associated with cell loss/atrophy.

Does low-dose lithium have utility in the treatment of mood disorders? While the data overall are not conclusive, there is considerable suggestive evidence to support such a contention. In this context, it is noteworthy that a recent demonstration of the potential usefulness of low-dose lithium was provided by Perlis et al.[127] Reanalyzing data from an earlier randomized, blinded study that determined that patients treated with low doses of lithium were more likely to relapse,[128] the newer study determined that it was the abrupt drop in lithium levels, rather than the absolute dose

Figure 4. Effects of Low-Dose Lithium Treatment on Bcl-2 Levels in Rat Frontal Cortex[a]
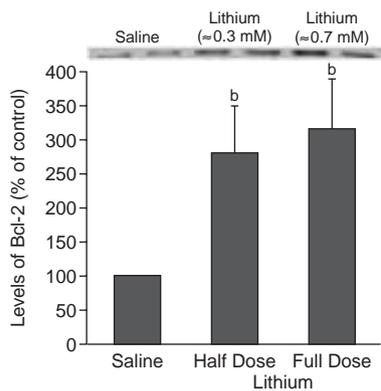
[a]Reprinted with permission from Manji et al.[43] Inbred male Wistar Kyoto rats were treated with Li_2CO_3 at "full dose" (resulting in plasma levels of ≈ 0.7 mM) or "half dose" (resulting in plasma levels of ≈ 0.3 mM) for 3 to 4 weeks. Immunoblotting of bcl-2 in frontal cortex was conducted using established methods with monoclonal antibodies directed against bcl-2. Treatment of rats with either full-dose or half-dose lithium for 3 to 4 weeks resulted in significant increases in the levels of bcl-2.
[b]$p < .05$ compared with control.

per se, that increased risk of new episodes. Patients who had been receiving similarly low doses before and after randomization were no more likely to relapse than those who were receiving high doses before and after randomization. Likewise, in a 12-month, double-blind, crossover study of high- and low-dose lithium, patients experienced more episodes during treatment with the low dose, but primarily in the first month following dose readjustment.[129] Longer studies have found low-dose lithium to be as effective as standard doses,[130,131] although there are conflicting reports.[132]

While the data are mixed, there is also substantial evidence suggesting that low-dose lithium is an effective potentiator of antidepressant action. In their review, Rouillon and Gorwood[133] discuss both case reports and larger trials and suggest that lithium augmentation should begin with a low dose, increasing only if necessary. It has been unequivocally established in controlled studies that about half of all treatment-refractory depressed patients respond to an addition of lithium to their ongoing antidepressant regimen. Although lithium potentiation is more efficacious in bipolar patients, it also has clear efficacy in the treatment of unipolar depressive patients. The lithium augmentation strategy derived from de Montigny and colleagues'[134] heuristic proposal that the enhancement of ascending presynaptic serotonergic function would translate into potentiation of antidepressant efficacy. However, the data reviewed here clearly show that lithium—even at lower doses—has major effects on neurotrophic signaling cascades, and it is our contention that these effects may also underlie its antidepressant potentiating efficacy. Finally, recent studies of elderly depressed patients suggest that low-dose lithium may be particularly useful in this population.[135] It is thus

clear that longitudinal studies using low-dose lithium as a "plasticity enhancer" are clearly warranted.

CONCLUDING REMARKS

As discussed, there is a considerable body of evidence to suggest both conceptually and experimentally that impairments in neuroplasticity and cellular resilience may play an important role in the pathophysiology of recurrent mood disorders. Furthermore, we contend that for many refractory depression patients, new drugs that simply mimic many "traditional" drugs that directly or indirectly alter neurotransmitter levels and those that bind to cell surface receptors may be of limited benefit. This is because such strategies implicitly assume that the target circuits are functionally intact and that altered synaptic activity will thus be transduced to modify the postsynaptic "throughput" of the system. However, the evidence presented here suggests that, in addition to neurochemical changes, many patients also have pronounced structural alterations (e.g., reduced spine density, neurite retraction, overall neuropil reductions) in critical neuronal circuits. Thus, optimal treatment may be attained only by providing both trophic and neurochemical support; the trophic support would be envisioned as enhancing and maintaining normal synaptic connectivity, thereby allowing the chemical signal to reinstate the optimal functioning of critical circuits necessary for normal affective functioning. Indeed, preliminary studies have suggested that regional structural brain changes in patients with mood disorders may be associated with not only illness severity/duration, but also altered treatment response.

Does the long-term administration of these agents actually retard disease- or affective episode–induced cell loss or atrophy? As articulated, the distinction between disease progression and affective episodes per se is an important one, since it is quite possible that the neurotrophic effects of lithium or valproate may even be independent of their ability to treat or prevent affective episodes. There are presently no longitudinal studies that we are aware of that can fully address this question, but this is clearly a very important and fundamental issue worthy of investigation. The findings that lithium administration increases brain NAA levels and gray matter volumes, as well as the cross-sectional study demonstrating "normalized" subgenual PFC volumes in lithium- and valproate-treated patients, do provide indirect support for such a contention. Longitudinal studies investigating the potential long-term neurotrophic effects (using serial volumetric MRI scans or MRS quantitation of regional NAA levels, for example) of mood stabilizers are clearly warranted. The evidence also suggests that early and potentially sustained treatment may be necessary to adequately prevent many of the deleterious long-term sequelae associated with mood disorders. While data suggest that hippocampal atrophy in depression is related to illness duration,[136] it is not presently clear if the

Table 3. Summary of the Differences in the Neurotrophic Properties of Antidepressants and Lithium: Evidence From Current Studies[a]

Antidepressants exert major effects on CREB and BDNF expression in rat hippocampus

Antidepressants *may* exert modest effects on ERK activation in rat brain

Lithium exerts major effects on ERK activation in rat frontal cortex and hippocampus

Lithium exerts major effects on bcl-2 in rat frontal cortex

Cross-sectional neuroimaging studies suggest that patients treated with chronic lithium or valproic acid do not show subgenual prefrontal cortex atrophy; patients treated with SSRIs show atrophy similar to that of untreated patients

Longitudinal studies show that chronic lithium increases the levels of NAA in areas of brain in bipolar patients; there are no similar published studies with valproic acid or antidepressants

Longitudinal studies show that chronic lithium increases gray matter volumes in bipolar patients; there are currently no similar published studies with antidepressants

[a]Reprinted with permission from Manji et al.[139]
Abbreviations: BDNF = brain-derived neurotrophic factor, CREB = cyclic adenosine monophosphate response element binding protein, ERK = extracellular signal-regulated kinase, NAA = *N*-acetyl-aspartate, SSRI = selective serotonin reuptake inhibitor.

volumetric and cellular changes that have been observed in other brain areas (most notably, frontal cortex) are related to affective episodes per se. Indeed, some studies have observed reduced gray matter volumes and enlarged ventricles in mood disorder patients at first onset of illness.[137,138] At this point, it is unclear if the cell death and atrophy in mood disorders occur because of the magnitude and duration of the biochemical perturbations, an enhanced vulnerability to the deleterious effects of these perturbations (due to genetic factors and/or early life events), or a combination thereof.

Given the large body of evidence supporting a neurotrophic and neuroprotective role for lithium and valproate, it is natural to wonder whether antidepressants may exert similar effects. In fact, antidepressants have been shown to increase the activity of the neuroprotective CREB transcription factor and to promote the expression of the neurotrophin BDNF. However, the similarity largely stops there; the mood stabilizers lithium and, to a certain extent, valproate exhibit neurotrophic and neuroprotective effects in a much broader array of measures than do antidepressants (highlighted in Table 3).

Finally, the data clearly suggest that a consideration of the potential utility of lithium and valproate in the treatment of other disorders associated with cell loss/atrophy is warranted. The activation of ERK MAP kinases and the up-regulation of bcl-2 are effects that lithium and valproate share with endogenous growth factors. Consistent with these effects, lithium has been demonstrated to exert neuroprotective effects in stroke models and Huntington's models, and against the toxicity produced by free radicals, human immunodeficiency virus, glutamate, β-amyloid, and 1-methyl-4-phenylpyridinium ion (MPP$^+$). While lithium and valproate are clearly not going to "cure" neurodegenerative disorders, it is our contention that their adjunctive use early in the course of these disorders has the potential to play an important role in slowing down disease progression by enhancing neuroplasticity and cellular resilience; clinical studies are thus clearly warranted.

Drug name: valproic acid (Depakene and others).

Disclosure of off-label usage: The authors of this article have determined that, to the best of their knowledge, valproic acid is not approved for neurodegenerative disorders.

REFERENCES

1. Johnson G. Lithium. Med J Aust 1984;141:595–601
2. Schou M. Bibliography on the biology and pharmacology of lithium: 6. Neuropsychobiology 1979;5:241–265
3. Goodwin FK, Jamison KR. Manic-Depressive Illness. New York, NY: Oxford University Press; 1990
4. Vestergaard P, Aagaard J. Five-year mortality in lithium-treated manic-depressive patients. J Affect Disord 1991;21:33–38
5. Muller-Oerlinghausen B, Ahrens B, Grof E, et al. The effect of long-term lithium treatment on the mortality of patients with manic-depressive and schizoaffective illness. Acta Psychiatr Scand 1992;86:218–222
6. Vestergaard P, Schou M. Lithium treatment in Aarhus, 1: prevalence. Pharmacopsychiatry 1989;22:99–100
7. Heit S, Nemeroff CB. Lithium augmentation of antidepressants in treatment-refractory depression. J Clin Psychiatry 1998;59(suppl 6):28–33
8. Reifman A, Wyatt RJ. Lithium: a brake in the rising cost of mental illness. Arch Gen Psychiatry 1980;37:385–388
9. Manji HK, Potter WZ, Lenox RH. Signal transduction pathways: molecular targets for lithium's actions. Arch Gen Psychiatry 1995;52:531–543
10. Schou M. Forty years of lithium treatment. Arch Gen Psychiatry 1997;54:9–13; discussion 14–15
11. Manji HK, Lenox RH. Signaling: cellular insights into the pathophysiology of bipolar disorder. Biol Psychiatry 2000;48:518–530
12. Mesulam MM. Neuroplasticity failure in Alzheimer's disease: bridging the gap between plaques and tangles. Neuron 1999;24:521–529
13. Manji H, Duman R. Impairments of neuroplasticity and cellular resilience in severe mood disorder: implications for the development of novel therapeutics. Psychopharmacol Bull 2001;35:5–49
14. Manji HK, Drevets WC, Charney DS. The cellular neurobiology of depression. Nat Med 2001;7:541–547
15. Soares JC, Mann JJ. The functional neuroanatomy of mood disorders. J Psychiatr Res 1997;31:393–432
16. Drevets WC. Functional anatomical abnormalities in limbic and prefrontal cortical structures in major depression. Prog Brain Res 2000;126:413–431
17. Drevets WC. Neuroimaging and neuropathological studies of depression: implications for the cognitive-emotional features of mood disorders. Curr Opin Neurobiol 2001;11:240–249
18. Strakowski SM, Adler CM, DelBello MP. Volumetric MRI studies of mood disorders: do they distinguish unipolar and bipolar disorder? Bipolar Disord 2002;4:80–88
19. Beyer JL, Krishnan KR. Volumetric brain imaging findings in mood disorders. Bipolar Disord 2002;4:89–104
20. Lenox RH, Gould TD, Manji HK. Endophenotypes in bipolar disorder. Am J Med Genet 2002;114:391–406
21. Carmelli D, Reed T, DeCarli C. A bivariate genetic analysis of cerebral white matter hyperintensities and cognitive performance in elderly male twins. Neurobiol Aging 2002;23:413–420
22. Taylor WD, Payne ME, Krishnan KR, et al. Evidence of white matter tract disruption in MRI hyperintensities. Biol Psychiatry 2001;50:179–183
23. Stoll AL, Renshaw PF, Yurgelun-Todd DA, et al. Neuroimaging in bipolar disorder: what have we learned? Biol Psychiatry 2000;48:505–517
24. Rajkowska G. Cell pathology in bipolar disorder. Bipolar Disord 2002;4:105–116
25. Bowley M, Drevets W, Ongur D, et al. Low glial numbers in the amygdala and entorhinal cortex in mood disorder. Biol Psychiatry 2002;51:97S–98S
26. Cotter D, Landau S, Beasley C, et al. The density and spatial distribution of GABAergic neurons, labelled using calcium binding proteins, in the anterior cingulate cortex in major depressive disorder, bipolar disorder, and schizophrenia. Biol Psychiatry 2002;51:377–386
27. Cotter D, Mackay D, Chana G, et al. Reduced neuronal size and glial cell density in area 9 of the dorsolateral prefrontal cortex in subjects with major depressive disorder. Cereb Cortex 2002;12:386–394

28. Miguel-Hidalgo J, Rajkowska G. Reduction of glia in the prefrontal cortex: a hypothesis of its role in the pathophysiology of major depressive disorder [abstract]. Biol Psychiatry 2002;51:98S

29. Cotter DR, Pariante CM, Everall IP. Glial cell abnormalities in major psychiatric disorders: the evidence and implications. Brain Res Bull 2001;55: 585–595

30. Coyle JT, Schwarcz R. Mind glue: implications of glial cell biology for psychiatry. Arch Gen Psychiatry 2000;57:90–93

31. Ongur D, Drevets WC, Price JL. Glial reduction in the subgenual prefrontal cortex in mood disorders. Proc Natl Acad Sci U S A 1998;95:13290–13295

32. Rajkowska G, Miguel-Hidalgo JJ, Wei J, et al. Morphometric evidence for neuronal and glial prefrontal cell pathology in major depression. Biol Psychiatry 1999;45:1085–1098

33. Ullian EM, Sapperstein SK, Christopherson KS, et al. Control of synapse number by glia. Science 2001;291:657–661

34. Hyman SE, Nestler EJ. Initiation and adaptation: a paradigm for understanding psychotropic drug action. Am J Psychiatry 1996;153:151–162

35. Wang JF, Young LT. Differential display PCR reveals increased expression of 2',3'-cyclic nucleotide 3'-phosphodiesterase by lithium. FEBS Lett 1996;386: 225–229

36. Lenox RH, McNamara RK, Papke RL, et al. Neurobiology of lithium: an update. J Clin Psychiatry 1998;59(suppl 6):37–47

37. Jope RS, Song L. AP-1 and NF-kappaB stimulated by carbachol in human neuroblastoma SH-SY5Y cells are differentially sensitive to inhibition by lithium. Brain Res Mol Brain Res 1997;50:171–180

38. Ozaki N, Chuang DM. Lithium increases transcription factor binding to AP-1 and cyclic AMP-responsive element in cultured neurons and rat brain. J Neurochem 1997;69:2336–2344

39. Asghari V, Wang JF, Reiach JS, et al. Differential effects of mood stabilizers on Fos/Jun proteins and AP-1 DNA binding activity in human neuroblastoma SH-SY5Y cells. Brain Res Mol Brain Res 1998;58:95–102

40. Chen G, Yuan PX, Jiang YM, et al. Valproate robustly enhances AP-1 mediated gene expression. Brain Res Mol Brain Res 1999;64:52–58

41. Ikonomov OC, Manji HK. Molecular mechanisms underlying mood stabilization in manic-depressive illness: the phenotype challenge. Am J Psychiatry 1999;156:1506–1514

42. Chen G, Zeng WZ, Yuan PX, et al. The mood-stabilizing agents lithium and valproate robustly increase the levels of the neuroprotective protein bcl-2 in the CNS. J Neurochem 1999;72:879–882

43. Manji HK, Moore GJ, Chen G. Lithium up-regulates the cytoprotective protein bcl-2 in the CNS in vivo: a role for neurotrophic and neuroprotective effects in manic depressive illness. J Clin Psychiatry 2000;61(suppl 9):82–96

44. Manji HK, Moore GJ, Chen G. Lithium at 50: have the neuroprotective effects of this unique cation been overlooked? Biol Psychiatry 1999;46: 929–940

45. Chen G, Rajkowska G, Du F, et al. Enhancement of hippocampal neurogenesis by lithium. J Neurochem 2000;75:1729–1734

46. Chen RW, Chuang DM. Long term lithium treatment suppresses p53 and Bax expression but increases Bcl-2 expression: a prominent role in neuroprotection against excitotoxicity. J Biol Chem 1999;274:6039–6042

47. Lu R, Song L, Jope RS. Lithium attenuates p53 levels in human neuroblastoma SH-SY5Y cells. Neuroreport 1999;10:1123–1125

48. Merry DE, Korsmeyer SJ. Bcl-2 gene family in the nervous system. Annu Rev Neurosci 1997;20:245–267

49. Adams JM, Cory S. The Bcl-2 protein family: arbiters of cell survival. Science 1998;281:1322–1326

50. Bruckheimer EM, Cho SH, Sarkiss M, et al. The Bcl-2 gene family and apoptosis. Adv Biochem Eng Biotechnol 1998;62:75–105

51. Gorman AM, Ceccatelli S, Orrenius S. Role of mitochondria in neuronal apoptosis. Dev Neurosci 2000;22:348–358

52. Chen DF, Tonegawa S. Why do mature CNS neurons of mammals fail to reestablish connections following injury? functions of bcl-2. Cell Death Differ 1998;5:816–822

53. Holm KH, Cicchetti F, Bjorklund L, et al. Enhanced axonal growth from fetal human bcl-2 transgenic mouse dopamine neurons transplanted to the adult rat striatum. Neuroscience 2001;104:397–405

54. Chierzi S, Strettoi E, Cenni MC, et al. Optic nerve crush: axonal responses in wild-type and bcl-2 transgenic mice. J Neurosci 1999;19:8367–8376

55. DeVries AC, Joh HD, Bernard O, et al. Social stress exacerbates stroke outcome by suppressing Bcl-2 expression. Proc Natl Acad Sci U S A 2001;98: 11824–11828

56. Watson SJ, Akil H. Gene chips and arrays revealed: a primer on their power and their uses. Biol Psychiatry 1999;45:533–543

57. Nadon R, Shoemaker J. Statistical issues with microarrays: processing and analysis. Trends Genet 2002;18:265–271

58. Yang F, Li X, Sharma M, et al. Linking beta-catenin to androgen-signaling pathway. J Biol Chem 2002;277:11336–11344

59. Liang P. A decade of differential display. Biotechniques 2002;33:338–344, 346

60. Lee ML, Kuo FC, Whitmore GA, et al. Importance of replication in microarray gene expression studies: statistical methods and evidence from repetitive cDNA hybridizations. Proc Natl Acad Sci U S A 2000;97:9834–9839

61. Alizadeh AA, Ross DT, Perou CM, et al. Towards a novel classification of human malignancies based on gene expression patterns. J Pathol 2001;195: 41–52

62. Yuan PX, Huang LD, Jiang YM, et al. The mood stabilizer valproic acid activates mitogen-activated protein kinases and promotes neurite growth. J Biol Chem 2001;276:31674–31683

63. Chen G, Huang LD, Jiang YM, et al. The mood-stabilizing agent valproate inhibits the activity of glycogen synthase kinase-3. J Neurochem 1999;72: 1327–1330

64. Yuan PX, Chen G, Huang LD, et al. Lithium stimulates gene expression through the AP-1 transcription factor pathway. Brain Res Mol Brain Res 1998;58:225–230

65. Tseng GC, Oh MK, Rohlin L, et al. Issues in cDNA microarray analysis: quality filtering, channel normalization, models of variations and assessment of gene effects. Nucleic Acids Res 2001;29:2549–2557

66. Hall AC, Brennan A, Goold RG, et al. Valproate regulates GSK-3-mediated axonal remodeling and synapsin 1 clustering in developing neurons. Mol Cell Neurosci 2002;20:257–270

67. Hall AC, Lucas FR, Salinas PC. Axonal remodeling and synaptic differentiation in the cerebellum is regulated by WNT-7a signaling. Cell 2000;100: 525–535

68. Lucas FR, Salinas PC. WNT-7a induces axonal remodeling and increases synapsin I levels in cerebellar neurons. Dev Biol 1997;192:31–44

69. Poo MM. Neurotrophins as synaptic modulators. Nat Rev Neurosci 2001; 2:24–32

70. Fryer RH, Kaplan DR, Feinstein SC, et al. Developmental and mature expression of full-length and truncated TrkB receptors in the rat forebrain. J Comp Neurol 1996;374:21–40

71. Gonzalez M, Ruggiero FP, Chang Q, et al. Disruption of Trkb-mediated signaling induces disassembly of postsynaptic receptor clusters at neuromuscular junctions. Neuron 1999;24:567–583

72. Mamounas LA, Blue ME, Siuciak JA, et al. Brain-derived neurotrophic factor promotes the survival and sprouting of serotonergic axons in rat brain. J Neurosci 1995;15:7929–7939

73. Goggi J, Pullar IA, Carney SL, et al. Modulation of neurotransmitter release induced by brain-derived neurotrophic factor in rat brain striatal slices in vitro. Brain Res 2002;941:34–42

74. Schinder AF, Berninger B, Poo M. Postsynaptic target specificity of neurotrophin-induced presynaptic potentiation. Neuron 2000;25:151–163

75. Drake CT, Milner TA, Patterson SL. Ultrastructural localization of full-length trkB immunoreactivity in rat hippocampus suggests multiple roles in modulating activity-dependent synaptic plasticity. J Neurosci 1999;19: 8009–8026

76. Riccio A, Ahn S, Davenport CM, et al. Mediation by a CREB family transcription factor of NGF-dependent survival of sympathetic neurons. Science 1999;286:2358–2361

77. Bonni A, Brunet A, West AE, et al. Cell survival promoted by the Ras-MAPK signaling pathway by transcription-dependent and -independent mechanisms. Science 1999;286:1358–1362

78. Chen G, Einat H, Yuan P, et al. Evidence for the involvement of the MAPK/ ERK signaling pathway in mood modulation [abstract]. Biol Psychiatry 2002;51:368s

79. Haugaard ES, Mickel RA, Haugaard N. Actions of lithium ions and insulin on glucose utilization, glycogen synthesis and glycogen synthase in the isolated rat diaphragm. Biochem Pharmacol 1974;23:1675–1685

80. Klein PS, Melton DA. A molecular mechanism for the effect of lithium on development. Proc Natl Acad Sci U S A 1996;93:8455–8459

81. Woodgett JR. Judging a protein by more than its name: GSK-3. Sci STKE 2001;2001:RE12

82. Ryves WJ, Harwood AJ. Lithium inhibits glycogen synthase kinase-3 by competition for magnesium. Biochem Biophys Res Commun 2001;280: 720–725

83. Phiel CJ, Klein PS. Molecular targets of lithium action. Annu Rev Pharmacol Toxicol 2001;41:789–813

84. Gould TD, Manji HK. The Wnt signaling pathway in bipolar disorder. Neuroscientist 2002;8:497–511

85. Li X, Bijur GN, Jope RS. Glycogen synthase kinase-3beta, mood stabilizers, and neuroprotection. Bipolar Disord 2002;4:137–144

86. Manji HK, Moore GJ, Chen G. Clinical and preclinical evidence for the neurotrophic effects of mood stabilizers: implications for the pathophysiology

and treatment of manic-depressive illness. Biol Psychiatry 2000;48: 740–754

87. Lucas FR, Goold RG, Gordon-Weeks PR, et al. Inhibition of GSK-3beta leading to the loss of phosphorylated MAP-1B is an early event in axonal remodelling induced by WNT-7a or lithium. J Cell Sci 1998;111:1351–1361

88. Hilfiker S, Pieribone VA, Czernik AJ, et al. Synapsins as regulators of neurotransmitter release. Philos Trans R Soc Lond B Biol Sci 1999;354: 269–279

89. Kao HT, Song HJ, Porton B, et al. A protein kinase A-dependent molecular switch in synapsins regulates neurite outgrowth. Nat Neurosci 2002;5: 431–437

90. Takashima A, Noguchi K, Sato K, et al. Tau protein kinase I is essential for amyloid beta-protein-induced neurotoxicity. Proc Natl Acad Sci U S A 1993;90:7789–7793

91. Pap M, Cooper GM. Role of glycogen synthase kinase-3 in the phosphatidylinositol 3-kinase/Akt cell survival pathway. J Biol Chem 1998; 273:19929–19932

92. Crowder RJ, Freeman RS. Glycogen synthase kinase-3 beta activity is critical for neuronal death caused by inhibiting phosphatidylinositol 3-kinase or Akt but not for death caused by nerve growth factor withdrawal. J Biol Chem 2000;275:34266–34271

93. Hetman M, Cavanaugh JE, Kimelman D, et al. Role of glycogen synthase kinase-3beta in neuronal apoptosis induced by trophic withdrawal. J Neurosci 2000;20:2567–2574

94. Culbert AA, Brown MJ, Frame S, et al. GSK-3 inhibition by adenoviral FRAT1 overexpression is neuroprotective and induces Tau dephosphorylation and beta-catenin stabilisation without elevation of glycogen synthase activity. FEBS Lett 2001;507:288–294

95. Martinek S, Inonog S, Manoukian AS, et al. A role for the segment polarity gene shaggy/GSK-3 in the Drosophila circadian clock. Cell 2001; 105:769–779

96. Klemfuss H. Rhythms and the pharmacology of lithium. Pharmacol Ther 1992;56:53–78

97. Wager-Smith K, Kay SA. Circadian rhythm genetics: from flies to mice to humans. Nat Genet 2000;26:23–27

98. Reppert SM, Weaver DR. Molecular analysis of mammalian circadian rhythms. Annu Rev Physiol 2001;63:647–676

99. Cohen P, Frame S. The renaissance of GSK3. Nat Rev Mol Cell Biol 2001;2:769–776

100. Maccioni RB, Munoz JP, Barbeito L. The molecular bases of Alzheimer's disease and other neurodegenerative disorders. Arch Med Res 2001;32: 367–381

101. Volonte C, Rukenstein A. Lithium chloride promotes short-term survival of PC12 cells after serum and NGF-deprivation. Lithium 1993;4:211–219

102. D'Mello SR, Anelli R, Calissano P. Lithium induces apoptosis in immature cerebellar granule cells but promotes survival of mature neurons. Exp Cell Res 1994;211:332–338

103. Li R, Shen Y, El-Mallakh RS. Lithium protects against ouabain-induced cell death. Lithium 1994;5:211–216

104. Alvarez G, Munoz-Montano JR, Satrustegui J, et al. Lithium protects cultured neurons against beta-amyloid-induced neurodegeneration. FEBS Lett 1999;453:260–264

105. Inouye M, Yamamura H, Nakano A. Lithium delays the radiation-induced apoptotic process in external granule cells of mouse cerebellum. J Radiat Res (Tokyo) 1995;36:203–208

106. Pascual T, Gonzalez JL. A protective effect of lithium on rat behaviour altered by ibotenic acid lesions of the basal forebrain cholinergic system. Brain Res 1995;695:289–292

107. Grignon S, Levy N, Couraud F, et al. Tyrosine kinase inhibitors and cycloheximide inhibit Li+ protection of cerebellar granule neurons switched to non-depolarizing medium. Eur J Pharmacol 1996;315:111–114

108. Chuang DM, Chen RW, Chalecka-Franaszek E, et al. Neuroprotective effects of lithium in cultured cells and animal models of diseases. Bipolar Disord 2002;4:129–136

109. Bijur GN, De Sarno P, Jope RS. Glycogen synthase kinase-3beta facilitates staurosporine- and heat shock-induced apoptosis: protection by lithium. J Biol Chem 2000;275:7583–7590

110. Arendt T, Lehmann K, Seeger G, et al. Synergistic effects of tetrahydroaminoacridine and lithium on cholinergic function after excitotoxic basal forebrain lesions in rat. Pharmacopsychiatry 1999;32:242–247

111. Sparapani M, Virgili M, Ortali F, et al. Effects of chronic lithium treatment on ornithine decarboxylase induction and excitotoxic neuropathology in the rat. Brain Res 1997;765:164–168

112. Nonaka S, Chuang DM. Neuroprotective effects of chronic lithium on focal cerebral ischemia in rats. Neuroreport 1998;9:2081–2084

113. Wei H, Qin ZH, Senatorov VV, et al. Lithium suppresses excitotoxicity-induced striatal lesions in a rat model of Huntington's disease. Neuroscience 2001;106:603–612

114. Chen G, Hasanat KA, Bebchuk JM, et al. Regulation of signal transduction pathways and gene expression by mood stabilizers and antidepressants. Psychosom Med 1999;61:599–617

115. Chalecka-Franaszek E, Chuang DM. Lithium activates the serine/threonine kinase Akt-1 and suppresses glutamate-induced inhibition of Akt-1 activity in neurons. Proc Natl Acad Sci U S A 1999;96:8745–8750

116. Datta SR, Dudek H, Tao X, et al. Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. Cell 1997;91:231–241

117. Kempermann G, Gage FH. Experience-dependent regulation of adult hippocampal neurogenesis: effects of long-term stimulation and stimulus withdrawal. Hippocampus 1999;9:321–332

118. Chen DF, Schneider GE, Martinou JC, et al. Bcl-2 promotes regeneration of severed axons in mammalian CNS. Nature 1997;385:434–439

119. Drevets WC. Neuroimaging studies of mood disorders. Biol Psychiatry 2000;48:813–829

120. Tsai G, Coyle JT. N-acetylaspartate in neuropsychiatric disorders. Prog Neurobiol 1995;46:531–540

121. Moore GJ, Bebchuk JM, Hasanat K, et al. Lithium increases N-acetylaspartate in the human brain: in vivo evidence in support of bcl-2's neurotrophic effects? Biol Psychiatry 2000;48:1–8

122. Moore GJ, Bebchuk JM, Wilds IB, et al. Lithium-induced increase in human brain grey matter. Lancet 2000;356:1241–1242

123. Moore G, Rajarethinam R, Cortese B, et al. Regionally specific increases in human brain gray matter with chronic lithium treatment [abstract]. Presented at the 31st annual meeting of the Society for Neuroscience; Nov 11, 2001; San Diego, Calif

124. Nonaka S, Hough CJ, Chuang DM. Chronic lithium treatment robustly protects neurons in the central nervous system against excitotoxicity by inhibiting N-methyl-D-aspartate receptor-mediated calcium influx. Proc Natl Acad Sci U S A 1998;95:2642–2647

125. Ren M, Chen RW, Senatorov VV, et al. Acute lithium protects against brain injury in an ischemia/reperfusion model of rats [abstract]. Presented at the 30th annual meeting of the Society for Neuroscience; Nov 6, 2000; New Orleans, La

126. Hashimoto R, Hough C, Nakazawa T, et al. Lithium protection against glutamate excitotoxicity in rat cerebral cortical neurons: involvement of NMDA receptor inhibition possibly by decreasing NR2B tyrosine phosphorylation. J Neurochem 2002;80:589–597

127. Perlis RH, Sachs GS, Lafer B, et al. Effect of abrupt change from standard to low serum levels of lithium: a reanalysis of double-blind lithium maintenance data. Am J Psychiatry 2002;159:1155–1159

128. Gelenberg AJ, Kane JM, Keller MB, et al. Comparison of standard and low serum levels of lithium for maintenance treatment of bipolar disorder. N Engl J Med 1989;321:1489–1493

129. Waters B, Lapierre Y, Gagnon A, et al. Determination of the optimal concentration of lithium for the prophylaxis of manic-depressive disorder. Biol Psychiatry 1982;17:1323–1329

130. Abou-Saleh MT, Coppen A. The efficacy of low-dose lithium: clinical, psychological and biological correlates. J Psychiatr Res 1989;23:157–162

131. Coppen A, Abou-Saleh MT. Lithium therapy: from clinical trials to practical management. Acta Psychiatr Scand 1988;78:754–762

132. Prien RF, Caffey EM Jr. Relationship between dosage and response to lithium prophylaxis in recurrent depression. Am J Psychiatry 1976;133: 567–570

133. Rouillon F, Gorwood P. The use of lithium to augment antidepressant medication. J Clin Psychiatry 1998;59(suppl 5):32–39

134. de Montigny C, Cournoyer G, Morissette R, et al. Lithium carbonate addition in tricyclic antidepressant-resistant unipolar depression: correlations with the neurobiologic actions of tricyclic antidepressant drugs and lithium ion on the serotonin system. Arch Gen Psychiatry 1983;40: 1327–1334

135. Wilkinson D, Holmes C, Woolford J, et al. Prophylactic therapy with lithium in elderly patients with unipolar major depression. Int J Geriatr Psychiatry 2002;17:619–622

136. Sheline YI, Sanghavi M, Mintun MA, et al. Depression duration but not age predicts hippocampal volume loss in medically healthy women with recurrent major depression. J Neurosci 1999;19:5034–5043

137. Strakowski SM, Wilson DR, Tohen M, et al. Structural brain abnormalities in first-episode mania. Biol Psychiatry 1993;33:602–609

138. Hirayasu Y, Shenton ME, Salisbury DF, et al. Subgenual cingulate cortex volume in first-episode psychosis. Am J Psychiatry 1999;156:1091–1093

139. Manji HK, Quiroz JA, Sporn J, et al. Enhancing synaptic plasticity and cellular resilience to develop novel, improved therapeutics for difficult-to-treat depression. Biol Psychiatry. In press