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Neuroplasticity and cellular resilience in mood disorders

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Although mood disorders have traditionally been regarded as good prognosis diseases, a growing body of data suggests that the long-term outcome for many patients is often much less favorable than previously thought. Recent morphometric studies have been investigating potential structural brain changes in mood disorders, and there is now evidence from a variety of sources demonstrating significant reductions in regional CNS volume, as well as regional reductions in the numbers and/or sizes of glia and neurons. Furthermore, results from recent clinical and preclinical studies investigating the molecular and cellular targets of mood stabilizers and antidepressants suggest that a reconceptualization about the pathophysiology and optimal long-term treatment of recurrent mood disorders may be warranted. It is proposed that impairments of neuroplasticity and cellular resilience may underlie the pathophysiology of mood disorders, and further that optimal long-term treatment for these severe illnesses may only be achieved by the early and aggressive use of agents with neurotrophic/ neuroprotective effects. It is noteworthy that lithium, valproate and antidepressants indirectly regulate a number of factors involved in cell survival pathways including CREB, BDNF, bcl-2 and MAP kinases, and may thus bring about some of their delayed long-term beneficial effects via underappreciated neurotrophic effects. The development of novel treatments which more directly target molecules involved in critical CNS cell survival and cell death pathways have the potential to enhance neuroplasticity and cellular resilience, and thereby modulate the long-term course and trajectory of these devastating illnesses. *Molecular Psychiatry* (2000) **5,** 578–593.

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There is mounting evidence that recurrent mood disorders—once considered 'good prognosis diseases'—are, in fact, often very severe and life-threatening illnesses. Recurrent mood disorders can have devastating long-term effects, and the cost of these illnesses in terms of human suffering, productivity and health care is enormous. Suicide is the cause of death in 10–20% of individuals, and in addition to suicide, mood disorders are associated with many other deleterious health-related effects.[1–5] Indeed, a recent study which controlled for physical illness, smoking and alcohol consumption found that the magnitude of the increased mortality risk conferred by the presence of high depressive symptoms was similar to that of stroke and congestive heart failure.[5] Not surprisingly, the costs associated with disability and premature death represent an economic burden of tens of billions of dollars annually in the United States alone.[1,6,7] It is now recognized that, for many patients, the long-term outcome is often much less favorable than previously thought, with incomplete interepisode recovery, and a progressive decline in overall functioning observed.[1] Indeed, according to the Global Burden of Disease Study, mood disorders are among the leading causes of disability worldwide, and are likely to represent an increasingly greater health, societal, and economic problem in the coming years.[6,7]

Despite the devastating impact that these diseases have on the lives of millions worldwide, there is still a dearth of knowledge concerning their underlying etiology and pathophysiology. There is thus considerable excitement regarding recent cellular and molecular biological studies, which have identified critical molecules regulating signaling and neuroplasticty as potential long-term mediators of mood stabilization.

'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 CNS have been characterized as including alterations of dendritic function, synaptic remodeling, long-term potentiation (LTP), axonal sprouting, neurite extension, synaptogenesis, and even neurogenesis (see Mesulam for an excellent overview).[8] Although the poten-

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tial relevance of neuroplastic events for the pathophysiology of psychiatric disorders has been articulated for some time,[9] 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.[10,11] In this paper, we discuss the results from recent clinical and preclinical studies using diverse paradigms which suggest that a reconceptualization about the pathophysiology, course, and optimal long-term treatment of recurrent mood disorders may be warranted. Indeed, it has recently been proposed that impairments of neuroplasticity and cellular resilience may underlie the pathophysiology of mood disorders,[10,11] and further that optimal long-term treatment for these severe illnesses may only be achieved by the early and aggressive use of agents with neurotrophic/neuroprotective effects, irrespective of the primary, symptomatic treatment.[11] Such treatment modalities, via their effects on critical molecules involved in cell survival and cell death pathways, such as CREB, BDNF, Bcl-2, p53 and MAP kinases have the potential to enhance neuroplasticity and cellular resilience, and thereby modulate the long-term course and trajectory of these devastating illnesses.[10,11]

Evidence for cell death, cell atrophy, and impairments of cellular resilience in mood disorders

Volumetric brain imaging

Recent morphometric neuroimaging studies have demonstrated that, *in toto*, patients with both bipolar disorder (BPD) and major depressive disorder (MDD) display morphometric changes suggestive of cell loss and/or atrophy.[12–18] The preponderance of the evidence from recent volumetric neuroimaging studies suggests an enlargement of third and lateral ventricles, as well reduced gray matter volumes in the orbital and medial prefrontal cortex (PFC), the ventral striatum, and the mesiotemporal cortex in patients with mood disorders.[12–18] Reductions in frontal lobe volumes, and striking ~40% reductions in the mean gray matter volume in the region located ventral to the genu of the corpus callosum have recently been demonstrated in BPD depressives and familial unipolar depressives.[14] Additional studies suggest that these subgenual PFC gray matter volume reductions may be particularly evident in 'enriched' patient populations, namely those with positive family histories of mood disorders.[19] Reductions in the volume of the hippocampus have also been observed in subjects with a history of MDD, findings which may persist for up to decades after the depressive episodes have resolved.[16,17,20–22] Interestingly, the loss of hippocampal volume appears to be correlated with the total lifetime duration of MDD but not with the age of the patients,[17] leading to the suggestion that these changes may represent the sequellae of repeated and/or prolonged episodes of depression[23–25] (discussed in detail later). Lending support to the structural neuroimaging literature are multiple functional brain imaging studies which have shown abnormalities in metabolic rate and blood flow in these same areas in mood disorders (reviewed in Drevets *et al*).[15]

Magnetic resonance spectroscopy

Magnetic Resonance Spectroscopy (MRS) is a tool which provides a non-invasive window to brain neurochemistry, and has increasingly been utilized in the study of neuropsychiatric disorders. *N*-Acetyl-aspartate (NAA) is one of the many neurochemical compounds which can be quantitatively assessed via MRS. NAA is the predominant resonance in the proton MRS spectrum of the normal adult human brain and while the functional role of this amino acid has not been definitively determined,[26] NAA is a putative neuronal marker, localized to mature neurons and not found in mature glial cells, CSF, or blood.[27] A number of studies have now shown that initial abnormally low brain NAA measures may increase and even normalize with remission of CNS symptoms in disorders such as demyelinating disease, amyotrophic lateral sclerosis, mitochondrial encephalopathies, and HIV dementia.[27] NAA is synthesized within mitochondria, and inhibitors of the mitochondrial respiratory chain decrease NAA concentrations, effects which correlate with reductions in ATP and oxygen consumption.[28] Thus, NAA is now generally regarded as a measure of *neuronal viability and function*, rather than strictly a marker for neuronal loss, *per se* (for an excellent recent review of NAA see Tsai and Coyle).[27] In recent studies using high resolution spectroscopic imaging methods, Bertolino *et al*[29] and Frye *et al*[30] found decreased NAA levels bilaterally in the hippocampus of BPD subjects compared to controls. Decreased levels of NAA have also been found bilaterally in the dorsolateral prefrontal cortex (DLPFC) in BPD patients compared to healthy controls.[31] Together, these studies add neurochemical support to the contention that mood disorders are associated with regional neuronal loss and/or reductions in neuronal viability/function. There have also been a number of reports of abnormal brain high energy phosphate metabolism in mood disorder patients, most notably decreased phosphocreatine (PCr) and/or ATP levels,[32–38] as well as abnormal phospholipid metabolism (predominantly phosphomonoesters and phosphodiesters).[33,34,36,37,39,40] The most extensive series of studies investigating possible abnormalities in brain energy regulation in mood disorders have been conducted by Kato and associates. Consistent with the decreased PCr and ATP levels discussed above, this research group has also found low pH levels (measured indirectly via ^{31}P MRS) in mood disorder patients compared to normal controls,[33,34,41] observations which have led to the postulation that BPD may be associated with mitochondrial dysfunction.[42] In a follow-up study, dynamic aspects of brain energy metabolism were studied by examining alterations in PCr and intracellular pH in the occipital region before, during and after photic stimulation. Although a number of confounding methodological factors (most

notably ongoing medication use) preclude a definitive interpretation of the results, it is noteworthy that BPD patients with a history of lithium-resistance exhibited a pronounced decrease in PCr levels during photic stimulation compared to the lithium-responsive patients.[42] In view of lithium's robust effects on the critical cytoprotective protein bcl-2 (*vide infra*), these results raise the intriguing possibility that lithium-responsiveness is due, at least in part, to enhancement of CNS mitochondrial function and cellular resilience. However, it is of course quite possible that lithium exerts robust neurotrophic and neuroprotective effects which are quite distinct from the mechanisms by which it treats affective episodes *per se*. The fact that lithium-responsive patients are much more likely to have received long-term lithium treatment would thus account for the 'protection' against photic stimulation-induced PCr reductions.[42]

Postmortem morphometric findings

In addition to the accumulating neuroimaging evidence, several postmortem brain studies are now providing direct evidence for reductions in regional CNS volume, cell number and cell body size. Baumann and associates[43,44] reported reduced volumes of the left nucleus accumbens, the right putamen and bilateral pallidum externum in postmortem brain samples obtained from patients with unipolar MDD or BPD.

Several recent postmortem stereological studies of the prefrontal cortex have also demonstrated reduced regional volume, cell numbers and/or sizes. Morphometric analysis of the density and size of cortical neurons in the DLPFC and orbitofrontal cortices has revealed significant reductions in mood disorder patients as compared to control subjects.[45,46] The neuronal reductions were, however, more subtle than the corresponding glial alterations (*vide infra*), and were detected only when specific morphological size-types of neurons were analyzed in individual cortical layers. For example, marked reductions in the density of large neurons (corresponding to pyramidal glutamatergic excitatory neurons) were found in layers III and V of the DLPFC in BPD and MDD.[45] In other prefrontal regions such as rostral orbitofrontal cortex, the most prominent neuronal reductions in MDD are confined to layer II cells (mostly corresponding to non-pyramidal inhibitory local circuit neurons). Reductions in the density of specific populations of layer II non-pyramidal neurons containing the calcium-binding protein calretinin have also been reported in the anterior cingulate cortex in subjects with a history of mood disorders.[47]

Additional morphometric studies have also reported layer-specific reductions in interneurons in the anterior cingulate cortex,[48] and reductions in nonpyramidal neurons (~40% lower) in CA2 of the hippocampal formation in BPD subjects compared to controls.[49] Overall, the layer-specific cellular changes observed in several distinct brain regions, including the prefrontal cortex, anterior cingulate cortex and hippocampus suggest that multiple neuronal circuits underlie the neuropathology of mood disorders. This is not altogether surprising since the behavioral and physiological manifestations of the illnesses are complex and include cognitive, affective, motoric, and neurovegetative symptomatology, as well as alterations of circadian rhythms and neuroendocrine systems, and are thus undoubtedly mediated by networks of interconnected neurotransmitter systems and neural circuits.[50–52]

In addition to neuronal pathology, unexpected reductions in glial cell number and density have also recently been found in postmortem brains of both MDD and BPD patients. Marked decreases in overall and laminar (layers III–IV) glial cell packing densities were found in subjects with MDD compared to nonpsychiatric control subjects.[45] Comparable reductions in glial densities were also detected in DLPFC from subjects with BPD.[46,53,54] Further immunohistochemical examination of PFC glial cells in MDD revealed that the reductions in the population of astroglial cells account, at least in part, for the global glial deficit that has also been found in this disorder.[55] In BPD, however, it is possible that a different population of glial cells (oligodendroglia and/or microglia) may be involved in this pathology, since reductions in a different morphological type of glial cell were consistently observed in all cortical layers of DLPFC in BPD subjects.[46] An independent histological study of area sg24 located in the subgenual PFC also found striking reductions in glial cell numbers in patients with familial MDD (24% reduction) and BPD (41% reduction) as compared to controls.[56] This observation is consistent with this research group's neuroimaging report on reductions in cortical gray matter volume found in the same brain region in a similar diagnostic group. While these results are intriguing, further immunohistochemical and molecular studies are needed to definitively determine if the same types of glial cells are involved in the glial deficit that has been observed in both MDD and BPD, and if this glial loss occurs via similar mechanisms. There is a growing appreciation of the critical roles of glia in regulating synaptic glutamate levels, CNS energy homeostasis, liberation of trophic factors, and indeed the very existence of synaptic networks of neurons and glia,[57,58] all of which suggest that the prominent glial loss observed in MDD and BPD may be integral to the pathophysiology of the disorders, and worthy of further study.

Overall, the preponderance of the data from the neuroimaging studies and the growing body of postmortem evidence presents a convincing case that there is indeed a reduction in regional CNS volume, accompanied by atrophy and loss of cells in at least a subset of patients with mood disorders. It remains to be fully elucidated to what extent these findings represent neurodevelopmental abnormalities, disease progression which *fundamentally involves loss/atrophy of glia and neurons*, or the sequellae of the biochemical changes (for example, in glucocorticoid levels) accompanying repeated affective episodes *per se*. Furthermore, precisely which of the prominent region-specific reductions in cell density represent true cell loss,

rather than extensive atrophy of cell bodies and/or their processes has not yet been fully established (for further review, see Rajkowska[54]). Morphometric analyses of cell sizes and cortical and laminar thickness suggest that, in addition to cell atrophy, some cell loss *does* occur in the PFC in mood disorders. The reductions in neuronal densities are paralleled by smaller sizes of neuronal somatas and significant 12–15% decreases in cortical thickness observed in rostral and middle orbitofrontal cortex in MDD.[45] It is noteworthy that while there are some striking similarities in the morphological changes found in MDD and BPD, there are also some differences. For example, the density of *both* large neurons and small neurons is decreased in BPD, whereas in MDD the reductions in the large neurons is accompanied by increases in the population of small neurons, suggesting atrophy rather than cell loss.[46,54] In BPD, these decreases in the density of both large and small types of neurons strongly indicate neuronal loss rather than an exclusive diminution in neuronal size in this disorder (Figure 1).

Potential mechanisms underlying cell death and atrophy in mood disorders

Preclinical stress paradigms have been extensively utilized to study potentially relevant neurobiological determinants of mood disorders. One of the most consistent effects of stress on cellular morphology is atrophy of hippocampal neurons (for reviews see Refs 24,25,59). This atrophy is observed in the CA3 pyramidal neurons, occurs after 2–3 weeks of exposure to restraint stress or longer-term social stress, and can be reversible.[24,25,59] Atrophy of CA3 pyramidal neurons also occurs upon exposure to high levels of glucocorticoids, suggesting that activation of the HPA axis likely plays a major role in mediating the stress-induced atrophy.[24,25] The potential etiological role for hypercortisolemia in hippocampal atrophy also receives support from the recent clinical study demonstrating increases in hippocampal volume following surgical treatment (transsphenoidal microadenomectomy) in Cushing's disease, effects which were associated with the magnitude of the decrease in urinary free cortisol.[60] In addition to neuronal atrophy, more long-term exposure to stress (ie for several months) can also result in true *loss of hippocampal neurons*.[24] Furthermore, increasing evidence has shown that stress and glucocorticoids also reduce overall cellular resiliency, thereby making neurons more vulnerable to a variety of other insults, including excitatory amino acids, ischemia, and hypoglycemia.[24] Thus, recurrent stress (and presumably recurrent affective episodes) may lower the threshold for cell death/atrophy in response to a variety of other pathological events. To date, there is a dearth of knowledge regarding the deleterious effects of stress and glucocorticoids on other brain areas; however, it is possible that stress and glucocorticoids also influence the survival and atrophy of neurons in other brain regions. This possibility is supported by recent clinical studies demonstrating cerebral atrophy in Cushing's disease,[61] and smaller intracranial and cerebral volumes in abused children and adolescents with post-traumatic stress disorder (PTSD).[62]

A growing body of data has implicated enhanced glutamatergic neurotransmission (mediated via both NMDA and non-NMDA receptors) in stress-induced hippocampal atrophy and death.[25] Interestingly, recent evidence suggests that certain insult-induced elevations in intrasynaptic glutamate levels may arise more from impairment of glutamate uptake (by both the presynaptic glutamatergic neuron and by surrounding glia), rather than by enhanced glutamate release.[24,63] These findings are particularly noteworthy since chronic lithium has recently been demonstrated to *enhance* glutamate reuptake.[64] Increases in extracellular levels of glutamate have been demonstrated to produce sustained activation of NMDA, and non-NMDA ionotropic receptors, both of which can produce potentially toxic increases in the levels of intracellular Ca^{2+}. Recent studies have demonstrated that both the subcellular compartmentalization of Ca^{2+} and the *source* of the Ca^{2+} may be a greater determinant of neurotoxicity than the absolute intracellular Ca^{2+} levels *per se*.[24] Furthermore, there appear to be functional relationships between Ca^{2+} released from IP_3-sensitive endoplasmic reticulum (ER) stores, and mitochondrial Ca^{2+} uptake, suggesting a critical role for the anti-apoptotic protein bcl-2 in subcellular Ca^{2+} homeostasis.[65] In view of the potential toxic effects of elevated intracellular Ca^{2+}, it is noteworthy that studies have consistently revealed elevations in basal and stimulated intracellular Ca^{2+}

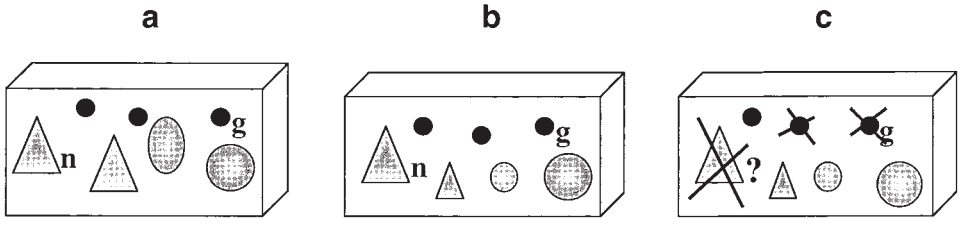

Figure 1 Patterns of cell change occurring in patients with mood disorders. (a) Normal cell number; (b) cell atrophy; (c) cell loss. Two major patterns of cell changes have been observed in postmortem brains of mood disorders patients. Cell atrophy (refers mainly to neurons—**n**, gray) is reported in DLPFC and orbitofrontal regions. This pathology is characterized by smaller cell body size and unchanged cell number compared to the brains of normal controls. By contrast, cell loss (refers mostly to glial cells—**g**, black) is reported in subgenual prefrontal cortex and possibly in DLPFC and orbitofrontal regions, and is characterized by reduced number of cells as compared to controls.

levels in peripheral cells of patients with BPD (discussed in Refs 66–68). Furthermore, a variety of missense, nonsense, frameshift and splicing mutations have recently been identified in the gene encoding the sarcoplasmic/endoplasmic reticulum calcium-pumping ATPase (SERCA2) in patients with Darier's disease (predominantly a skin disorder), many of whom exhibited neuropsychiatric (including affective) phenotypes.[69] These findings are intriguing since abnormalities of Ca^{2+} handling have also been observed when peripheral cells obtained from BPD patients have been treated with the SERCA inhibitor thapsigargin.[70] The potential role for abnormalities of Ca^{2+} signaling in the pathophysiology of BPD is also highlighted by the recent observations that the chronic administration of the most effective mood stabilizers, lithium and valproate (VPA), robustly increases the expression of two proteins known to play important roles in calcium sequestration. Thus, lithium has been demonstrated to robustly increase the levels of the major anti-apoptotic protein bcl-2 in several brain regions and in human neuroblastoma cells,[11,71–75] whereas chronic VPA has been demonstrated to increase the expression of the ER chaperone protein GRP78[76] (*vide infra*).

Another mechanism that could contribute to the deleterious effects of stress, as well as the cellular changes observed in mood disorders is the regulation of neurotrophic factors. Thus, immobilization-, footshock- and chronic unpredictable-stress all decrease brain derived neurotrophic factor (BDNF) expression in the hippocampus.[77,78] Since BDNF and other neurotrophic factors are necessary for the survival and function of neurons,[79,80] sustained reduction of these factors could markedly affect neuronal viability. The precise mechanisms underlying the stress-induced reductions in BDNF expression remain to be fully elucidated, but do not appear to involve glucocorticoids. The potential role of cytokines is worthy of investigation since interleukin-1β (IL-1 β), has been shown to contribute to the stress-induced impairment of LTP and age-related damage of hippocampus.[81] In this context, it is noteworthy that it has recently been suggested that brain cytokines, in particular interleukin-1β, may be involved in the pathophysiology and some of the somatic consequences of MDD.[4]

Programmed cell death/apoptosis

A mounting body of data suggests that programmed cell death or apoptosis may contribute to the loss of neurons observed in a variety of pathological conditions. There is a growing appreciation that for many cells, there is a very fine balance maintained between the levels and activities of pro- and anti-apoptotic factors, and that modest changes in these factors (potentially due to genetic, illness or insult-related factors) may profoundly affect cellular viability. A primary component of apoptosis is activation of a family of cysteine proteases (referred to as caspases) which degrade many proteins that are essential for cell survival.[82] It is now clear that the Bcl-2 family of proteins plays a critical role in regulating cellular survival.[83] This family consists of both anti-apoptotic (eg Bcl-2 and Bcl-XL) and pro-apoptotic members (eg Bax and Bad),[83] many of which are expressed in the rodent and mammalian CNS.[84]

Bcl-2 attenuates apoptosis by sequestering proforms of death-driving caspases, by preventing the release of mitochondrial apoptogenic factors into the cytoplasm, and by enhancing mitochondrial calcium uptake.[83,85] Increasing evidence suggests a critical role for the mitochondria in the process of apoptosis. 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.[86] One of the major mechanisms by which Bcl-2 appears to exert its protective effects against a variety of disparate insults is by acting on mitochondria to stabilize membrane integrity and to prevent opening of the permeability transition pore.[86] Additionally, a growing body of data suggests that bcl-2 also regulates calcium homeostasis in the ER.[87,88] In view of the mutations in the SERCA2 pump described above, it is noteworthy that studies have shown that bcl-2 can interact with SERCA and either maintain calcium uptake into the ER or reduce calcium efflux from the ER in cells treated with the SERCA inhibitor thapsigargin.[89,90] It is thus likely that bcl-2's major effects on calcium homeostasis play a critical role in its ability to protect neurons from a variety of insults both *in vitro* and *in vivo* (discussed in Refs 11,73,75,83–85 and references therein). A growing body of data is also showing that, in addition to its well established neuroprotective effects, bcl-2 may also exert independent *neurotrophic* effects. Thus, Bcl-2 overexpression has also been shown to promote *regeneration* of axons in the mammalian CNS,[91] to regulate neurite sprouting and outgrowth,[91] and to increase axonal growth rate,[92,93] effects which may all be independent of its anti-apoptotic effects. Importantly for the present discussion, bcl-2 has also been demonstrated to rescue cells from toxin-induced cell *atrophy*;[94] it has thus been convincingly argued that increasing CNS Bcl-2 levels may represent a very effective neurotrophic strategy to enhance cellular resiliency.[91]

It is now known that neurotrophic factors (such as BDNF) promote cell survival *largely by suppressing intrinsic, cellular apoptotic machinery*, rather than by inducing cell survival pathways.[79,95] This occurs via binding of these factors to specific membrane receptors and the regulation of two intracellular signal transduction pathways that are crucial in promoting neuronal survival—the mitogen activated protein (MAP) kinase cascade and the phosphotidylinositol-3 kinase (PI-3K)/Akt pathway[96,97] (Figure 2). Recent studies have demonstrated that the activation of the MAP kinase pathway can inhibit apoptosis by inducing the phosphorylation of Bad and increasing the expression of Bcl-2, the latter effect likely involving the cAMP response element binding protein (CREB).[98,99] Phosphorylation of Bad occurs via activation of a down-

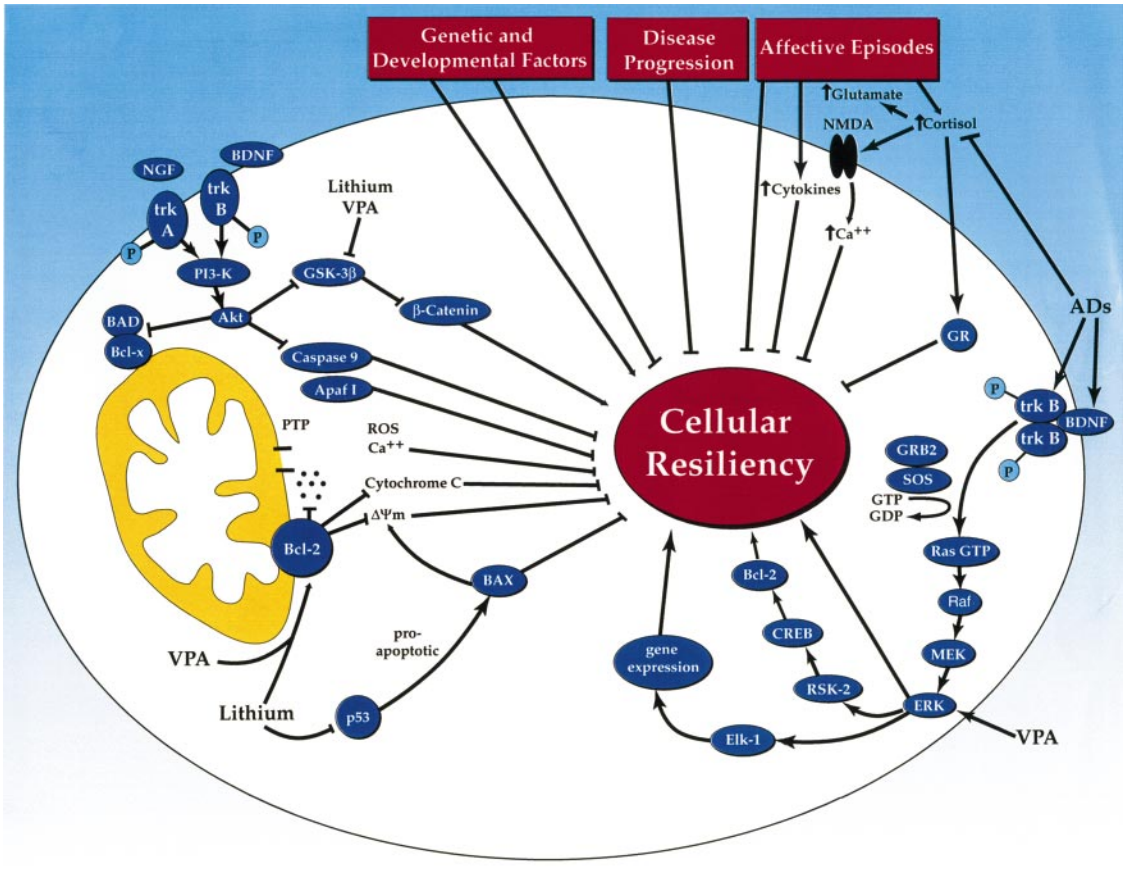

Figure 2 Cellular resilience in mood disorders. This figure depicts the multiple influences on neuroplasticity and cellular resilience in mood disorders. Genetic/neurodevelopmental factors, repeated affective episodes (and likely elevations of glucocorticoids) and illness progression may all contribute to the impairments of cellular resilience, volumetric reductions and cell death/atrophy observed in mood disorders. 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 AIF (apoptosis-inducing factor) into the cytoplasm, and by enhancing mitochondrial calcium uptake. 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; bcl-2 acts on mitochondria to stabilize membrane integrity and to prevent opening of the permeability transition pore. Lithium, via its effects on bcl-2 and p53 may exert effects on the mitochondrial permeability transition pore, a key event in cell death. Lithium and VPA also inhibit GSK-3β, biochemical effects shown to have neuroprotective effects. VPA also activates the ERK MAP kinase pathway, effects which may play a major role in neurotrophic effects and neurite outgrowth. Antidepressants regulate the expression of BDNF, and its receptor TrkB. Both TrkA and TrkB utilize the PI-3-kiase/Akt and ERK MAP kinase pathways to bring about their neurotrophic effects. The ERK MAP kinase cascade also increases the expression of bcl-2 via its effects on CREB. BDNF, brain derived neurotrophic receptor; trkB, tyrosine kinase receptor for BDNF; NGF, nerve growth factor; trkA, tyrosine kinase receptor for NGF; Bcl-2 and Bcl-x, anti-apoptotic members of the bcl-2 family; BAD and Bax, proapoptotic members of the bcl-2 family; GRB-2; scaffolding protein with *src* homology domains to coordinate MAP kinase signaling pathways; sos, son of sevenless—facilitates guanine nucleotide exchange; GAPs, GTPase activating proteins; Ras, Raf, MEK, ERK, Elk1, components of the ERK MAP kinase pathway; PTP, mitochondrial permeability transition pore; $\Delta\Psi_m$, mitochondrial inner transmembrane potential; CREB, cyclic AMP responsive element binding protein; Rsk-2, ribosomal S-6 kinase; ROS, reactive oxygen species; GR, glucocorticoid receptor.

stream target of the MAP kinase cascade, ribosomal S-6 kinase (Rsk). Rsk phosphorylates Bad and thereby promotes its inactivation. Activation of Rsk also mediates the actions of the MAP kinase cascade and neurotrophic factors on the expression of Bcl-2. Rsk can phosphorylate the cAMP response element binding protein (CREB) and this leads to induction of Bcl-2 gene expression (see Figure 2).

Do impairments in neurogenesis contribute to the cellular changes observed in mood disorders?

The preceding discussion has centered largely around the possibility that the regional reductions in cell numbers observed in mood disorders is primarily due to cell death. However, the demonstration that neurogenesis occurs in *the human brain* into senescence,[100]

raises the possibility that ongoing impairment of neurogenesis may also play a role. The greatest density of new cell birth is observed in the subventricular zone and the subgranular layer of the hippocampus, although a recent study has suggested that new neurons originating from the subventricular zone are found also in areas of association cortex of nonhuman primates.[101] Recent studies have shown that decreased neurogenesis occurs in response to both acute and chronic stress, effects which appear to be mediated by glucocorticoids.[102] Thus, it is an interesting possibility that the reduced hippocampal volumes that have been observed in conditions associated with elevated glucocorticoid levels (eg MDD, Cushing's, PTSD) may be due, at least in part, to an impairment of neurogenesis. At present, it is not clear to what extent ongoing neurogenesis may contribute to the appearance of new neurons in other brain regions, and if these newborn neurons are also regulated by glucocorticoids in a similar manner. Age-related increases in glucocorticoid levels have also been postulated to be responsible for the reduced rate of neurogenesis observed in aged mammals, since lowering of glucocorticoid levels in these animals restores neurogenesis to levels observed in younger animals.[103] These observations raise the intriguing possibility that CRF antagonists, currently being developed for the treatment of mood and anxiety disorders, may have particular utility in the treatment of elderly depressed patients.

Influence of antidepressant treatment on cell survival pathways

Elegant recent work by Duman and associates[10,104] has shown that factors involved in neuronal atrophy and survival may be the target of antidepressant treatments, observations which have led to the formulation of a heuristic molecular and cellular hypothesis of depression.[10] These investigators have demonstrated that chronic, but not acute, administration of different classes of antidepressants up-regulates the cAMP-CREB cascade.[105] This observation is particularly noteworthy since BDNF is known to be regulated by CREB,[106] and consistent with this, chronic antidepressant treatment increases the expression of BDNF in the rodent hippocampus.[107,108]

More recently, the influence of chronic antidepressant treatment on neurogenesis of hippocampal neurons has been examined.[109] Chronic, but not acute, antidepressant treatment was found to increase the number of new cells in the dentate gyrus granule cell layer. Furthermore, these effects were observed with different classes of antidepressants, but not with several other psychotropic medications investigated.[109] Consistent with their cellular effects, several reports support the hypothesis that chronic antidepressant treatment produces neurotrophic-like effects.[104] Thus, studies have demonstrated that AD treatment induces greater regeneration of catecholamine axon terminals in the cerebral cortex,[110] and at least one atypical antidepressant (tianeptine) has been demonstrated to attenuate stress-induced atrophy of hippocampal CA3 pyramidal neurons.[111] Additional studies are clearly needed to further characterize the neurotrophic/neuroprotective effects of antidepressants in other models of cell damage or atrophy. A recent electrophysiological study has also demonstrated that chronic ADs increase the field excitatory postsynaptics potentials connectivity in the dentate gyrus, effects which could represent an increase in synaptic connections and number of new neurons in the dentate gyrus granule cell layer.[112]

Neurotrophic and neuroprotective effects of lithium and vaproate

Lithium robustly upregulates the critical cytoprotective protein bcl-2

Recent mRNA RT-PCR Differential Display studies have led to the identification of a completely unexpected target for the actions of chronic lithium and valproate (VPA) in the frontal cortex (FCx)—the cytoprotective protein bcl-2.[11,71,73,75] Chronic treatment of rodents with 'therapeutic' doses of lithium or VPA was found to produce a *doubling* of bcl-2 levels in the FCx, effects which were primarily due to a marked increase in the number of bcl-2 immunoreactive cells in layers II and III of FCx (Figure 3). As discussed, these are the very same brain regions where the greatest neuronal changes have been observed in morphometric studies of mood disorder patients, and primate studies have indicated that neurons in the layers II–IV of the FCx are important sites for connections with other cortical regions, and major targets for subcortical input.[45] Chronic lithium also markedly increased the number of bcl-2 immunoreactive cells in the dentate gyrus and striatum of rats,[73] as well as in the hippocampus of C57BL/6 mice.[113] The fact that these intriguing effects of lithium represent direct cellular effects of the monovalent cation, rather than alterations in synaptic throughput or long-loop feedback pathways is suggested by the demonstration that chronic *in vitro* lithium also robustly increases the levels of bcl-2 in human neuroblastoma SH-SY5Y cells,[75] and in rat cerebellar granule cells.[72] Interestingly, not only does chronic lithium increase the expression of bcl-2, it also produces *reductions* in the levels of the *pro*-apoptotic protein p53 both in rat cerebellar granule cells[72] and human neuroblastoma SH-SY5Y cells.[114] Thus, overall the data clearly show that chronic lithium robustly increases the levels of the neuroprotective protein bcl-2 in areas of rodent FCx, 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 the pro-apoptotic protein p53.

Inhibition of glycogen synthase kinase 3β (GSK-3β) may also exert neuroprotective effects

In addition to bcl-2, another novel target for the actions of lithium has been identified in recent years. Thus, Klein and Melton[115] first demonstrated that lithium, at therapeutically relevant concentrations, is an inhibitor

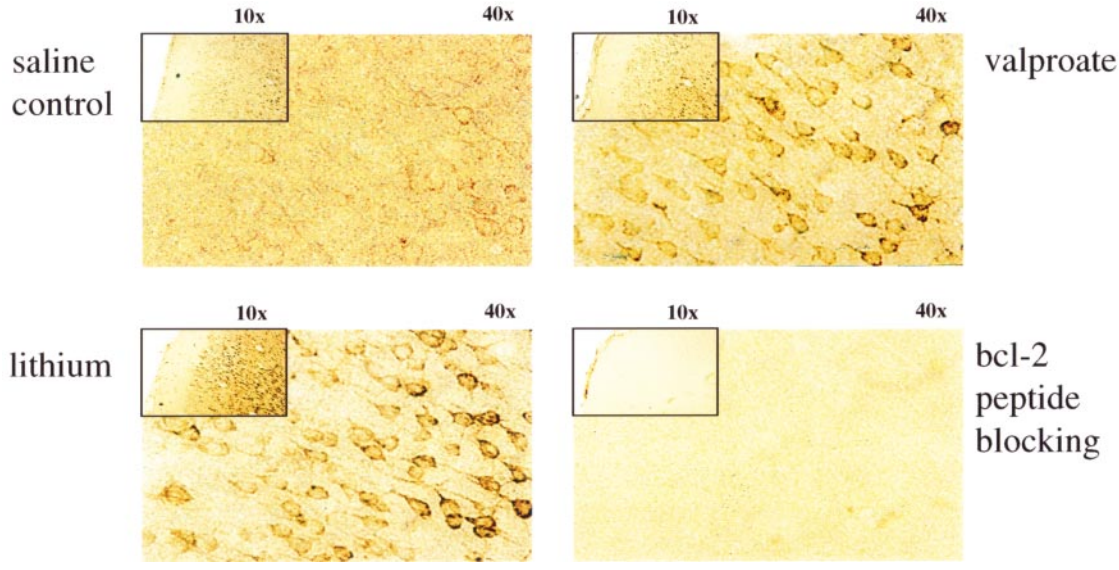

Figure 3 Chronic lithium and valproate robustly increase bcl-2 immunoreactive neurons in the frontal cortex. Male Wistar Kyoto rats were treated with either Li_2CO_3, valproate or saline by twice daily i.p. injections for 4 weeks. Rat brains were cut at 30 μm; serial sections were cut coronally through the anterior portion of the brain, mounted on gelatin-coated glass slides and were stained with thionin. The sections of the second and third sets were incubated free-floating for 3 days at 4°C in 0.01 M PBS containing a polyclonal antibody against bcl-2 (N-19, Santa Cruz Biotechnology, Santa Cruz, CA, USA 1:3000), 1% normal goat serum and 0.3% Triton X-100 (Sigma, St Louis, MO, USA). Subsequently, the immunoreaction product was visualized according to the avidin-biotin complex method. The figure shows immunohistochemical labeling of bcl-2 in layers II and III of frontal cortex in saline-, lithium- or valproate-treated rats. Blocking peptide shows the specificity of the antibody. Photographs were obtained with 40 × magnification. Modified and reproduced, with permission, from Reference 71.

of GSK3β. In addition to its critical roles in the developing CNS, GSK3β is now known to play an important role in the mature CNS, by regulating various cytoskeletal processes and long-term nuclear events via phosphorylation of c-jun, nuclear, translocation of β-catenin, and nuclear export of NF-ATc (reviewed in Refs 116–118). GSK-3β also regulates the phosphorylation of *tau* and beta-catenin, both of which have been implicated in certain types of disease-related neuronal death (discussed in Refs 73, 116, 117, 119, 120). Overexpression of GSK-3 has been shown to induce apoptosis of PC12 cells,[121] and to potentiate staurosporine-induced caspase activation.[122] Several recent studies have also found that inhibition of GSK-3β by lithium reduces *tau* phosphorylation, effects which likely also occur at therapeutically relevant lithium concentrations (see Jope for an excellent discussion).[118] Although many of the studies have utilized lithium concentrations in excess of those utilized therapeutically, the available data suggest that lithium, at concentrations of ~1 mM does, indeed, reduce *tau* phosphorylation.[118,123–125] Overall, the data suggest that in addition to bcl-2 upregulation, inhibition of GSK-3β by lithium may also afford protection against the cell death induced by various stimuli.[126] In view of the important role of GSK-3β in cell survival, a study was undertaken to determine if other mood stabilizers also regulate GSK3β. VPA was found to significantly inhibit GSK3β at therapeutically relevant concentrations, whereas carbamazepine was without any effect.[127] Consistent with GSK3β inhibition, VPA produced a robust time-dependent increase in both cytosolic and nuclear β-catenin levels in human neuroblastoma SH-SY5Y cells.[127] Another independent laboratory has recently also demonstrated that VPA increases β-catenin levels, and increases the expression of a reporter gene driven by β-catenin/LEF transcription factor (personal communication to HK Manji from PS Klein, March 2000). Most recently, it has been demonstrated that the chronic (3–4 week) administration of lithium or VPA also increases β-catenin levels in rodent brain (Chen and Manji, unpublished observations), compatible with inhibition of GSK3β during chronic *in vivo* administration of the agents under therapeutic paradigms.

Neuroprotective effects of lithium: compelling preclinical evidence

Lithium's robust effects of bcl-2 and GSK-3β *in the mature* CNS suggest that it may possess significant neuroprotective properties. Indeed, several studies which were conducted before the identification of bcl-2 or GSK-3β as targets for lithium's actions *had already demonstrated neuroprotective properties* of lithium.[128–134] The protective effects of lithium have been investigated in a number of *in vitro* studies of rat cerebellar granule cells, and lithium has been shown to protect against the deleterious effects of glutamate, NMDA receptor activation, low potassium, and toxic concentrations of anticonvulsants.[135,136] Lithium also protects PC12 cells from serum/nerve growth factor deprivation,[128] protects both PC12 cells and human

neuroblastoma SH-SY5Y cells from ouabain toxicity,[130] and protects SH-SY5Y cells from both thapsigargin (which mobilizes intracellular Ca^{2+}) and MPP^+-induced cell death.[76] Most recently, lithium has been shown to protect cultured neurons from beta amyloid-induced cell death,[131] and to protect against the deleterious effects of GSK-3β overexpression coupled to staurosporine addition.[122]

Even more impressive are the studies which have clearly demonstrated lithium's neuroprotective effects in the rodent brain *in vivo*. Lithium pretreatment has been shown to attenuate both the biochemical and behavioral manifestations of excitotoxic lesions of the cholinergic system,[133,137] and to attenuate the kainic acid-induced reduction in glutamate decarboxylase levels and [^{3}H]D-asparatate uptake.[138] 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 neurological deficits (abnormal posture and hemiplegia).[139] 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.[140] In addition to its effects on bcl-2 and GSK-3β, lithium's effects on other signaling pathways and transcription factors[51,118] 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.[141] Furthermore, it has been 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 clear demonstration of lithium-induced activation of Akt-1 in the CNS *in vivo*.

Lithium increases hippocampal neurogenesis

As discussed already, the seminal study by Erikkson and associates[100] has 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, likely via apoptosis.[142] In view of bcl-2's major neuroprotective and neurotrophic role, a study was undertaken to determine if lithium, administrated at therapeutically relevant concentrations, affects neurogenesis in the adult rodent brain. After treatment with lithium for 14 days, mice were administered single doses of BrdU (bromodeoxyuridine, a thymidine analog which 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,[113] unbiased, stereological 3-D cell counting was performed using a computer-assisted image analysis system, and revealed that chronic lithium administration *does*, indeed, increase in the number of BrdU positive cells in the dentate gyrus by ~25% (Figure 4).[113] 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 observed, and studies using bcl-2 transgenic animals are currently underway to delineate the role of bcl-2 overexpression in the enhanced hippocampal neurogenesis observed.

Valproate robustly activates the ERK MAP kinase pathway

VPA's effects on bcl-2 and GSK-3β suggest that this mood-stabilizer may also possess neuroprotective/neurotrophic properties. Additionally, as discussed, VPA also increases the expression of the molecular chaperone GRP78.[76] The ER chaperone protein GRP78 suppresses elevations of intracellular Ca^{2+} following exposure of neurons to glutamate, effects which appear to occur via suppression of Ca^{2+} from ryanodine-sensitive stores.[65] Although not as extensively studied as lithium, a growing body of data suggests that VPA does, indeed, exert neuroprotective effects.[75,143–145]

Although much recent evidence has also shown that VPA increases AP-1 DNA binding activity and AP-1 mediated gene expression,[146] the mechanisms underlying these effects have not been fully elucidated. In this context, MAP (mitogen activated protein) kinases play a key role in the regulation of the AP-1 family of transcription factors.[147] In view of the important role of MAP kinases in mediating long-term neuroplastic events, and in regulating AP-1 activity, a series of studies were undertaken to determine if VPA regulates MAP kinases. It was found that VPA robustly activates the ERK MAP kinase pathway, as well as ERK/Elk-1 mediated gene expression[113] (Figure 2). Since the ERK MAP kinases are known to mediate many of the effects of various neurotrophic factors (including nerve growth factor (NGF) and brain derived neurotrophic factor (BDNF)), and to promote neurite outgrowth,[96,148] VPA's effects on the morphology of SH-SY5Y cells were investigated in detail. Exposure of SH-SY5Y cells to VPA (1.0 mM) in serum-free media for 5 days not only resulted in robust neurite outgrowth, but also prominent growth cone formation, and marked increases in the levels of both GAP-43 (>3-fold increases) and bcl-2 (>5-fold increases).[149] In view of VPA's apparent trophic effects, human neuroblastoma SH-SY5Y cells were grown in the presence of therapeutic concentrations of VPA *without any additional neurotrophic factors*. Remarkably, cells grown only in the presence of VPA continued to grow well for >40 days. As discussed, a variety of neurotrophins activate the ERK pathway via cell surface tyrosine kinase receptors (eg trkB), and ERK pathways are known to play a major role in neurotrophin-induced cell differentiation and neurite growth.[96,148] It is thus noteworthy that VPA activates the ERK MAP kinase pathway and promotes neurite growth in SH-SY5Y cells, effects which are characteristic of *endogeneous neurotrophic factors*.

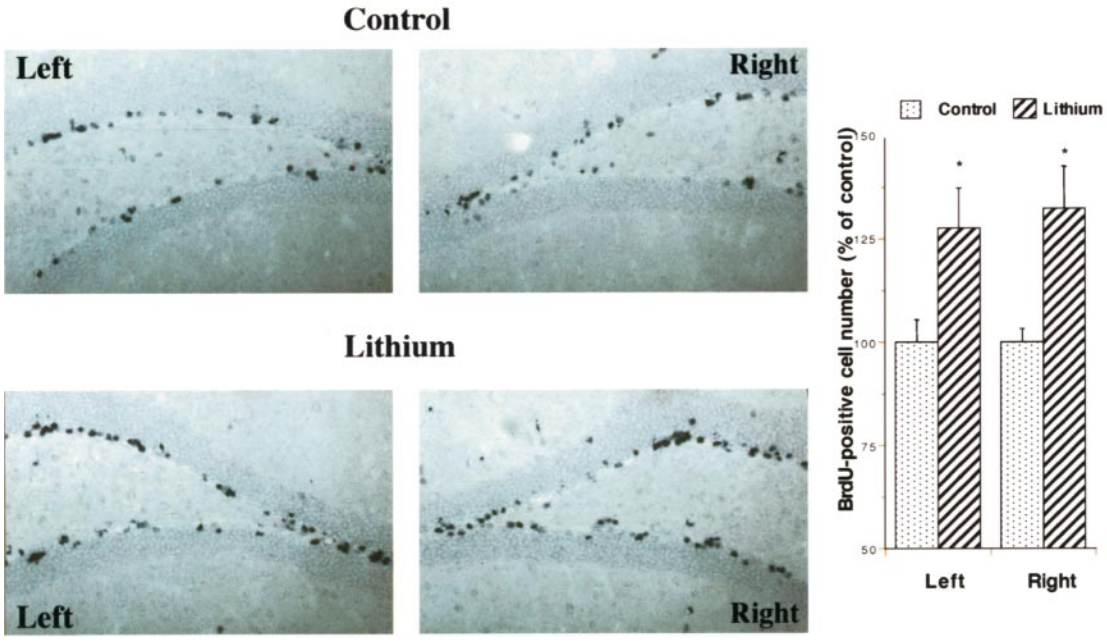

Figure 4 Chronic lithium increases hippocampal neurogenesis. C57BL/6 mice were treated with lithium for 14 days, and then received once daily BrdU injections for 12 consecutive days while lithium treatment continued. Twenty-four hours after last injection, the brains were processed for BrdU immunohistochemistry. Cell counts were performed in the hippocampal dentate gyrus at three levels along the dorsoventral axis in all the animals. BrdU-positive cells were counted using unbiased sterological methods. Chronic lithium produced a significant 25% increase in BrdU immunolabeling in both right and left dentate gyrus ($*P < 0.05$). Modified and reproduced, with permission, from Reference 113.

Can the neurotrophic effects of mood stabilizers be demonstrated in *the human brain?*

While the body of preclinical data demonstrating neurotrophic and neuroprotective effects of lithium is striking, considerable caution must clearly be exercised in extrapolating to the clinical situation with humans. In view of lithium and VPA's robust effects on the levels of the cytoprotective protein bcl-2 in the frontal cortex, Drevets and associates have re-analyzed their data demonstrating ~40% reductions in subgenual PFC volumes in familial mood disorder subjects. Consistent with neurotrophic/neuroprotective effects of lithium and VPA, they found that the patients treated with chronic lithium or VPA exhibited subgenual PFC volumes which were significantly higher than the volumes in non lithium- or VPA-treated patients, and not significantly different from controls (personal communication from W Drevets to HK Manji, July 1999).

A longitudinal clinical study was recently undertaken to determine if lithium also exerts neurotrophic/neuroprotective effects in the *human brain in vivo*. Proton magnetic resonance spectroscopy (MRS) was utilized to quantitate NAA levels longitudinally. As discussed, NAA is believed to represent a putative marker of neuronal viability and has been utilized to follow the course of neurodegenerative disorders.[27] After extensive validation of this method for longitudinal *in vivo* measurement, regional NAA concentrations were measured in BPD patients at baseline (after a >2-week medication washout), and again after 4 weeks of lithium at therapeutic doses. Chronic Li administration was found to significantly increase NAA concentration, and furthermore, a striking ~0.97 correlation between lithium-induced NAA increases and regional voxel gray matter content was observed.[150] 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 exerts *neurotrophic effects* in humans.

In a follow-up study to the NAA findings, it was hypothesized that, in addition to increasing functional neurochemical markers of neuronal viability, lithium-induced increases in bcl-2 would also lead to neuropil increases, and thus to increased brain gray matter volume in BPD patients. In this clinical research investigation, brain tissue volumes were examined using high resolution three dimensional MRI and validated quantitative brain tissue segmentation methodology to identify and quantify the various components by volume, including total brain white and gray matter content. Measurements were made at baseline and then repeated after 4 weeks of lithium at therapeutic doses. This study revealed an extraordinary finding that chronic lithium significantly increases *total gray matter content* in the human brain of the patients with MDI (Figure 5).[151] No significant changes were observed in brain white matter volume, or in quantitative measures of regional cerebral water content, thereby providing strong evidence that the observed increases in gray matter content are likely due to neurotrophic effects as opposed to any possible cell swelling and/or osmotic effects associated with lithium treatment. A finer

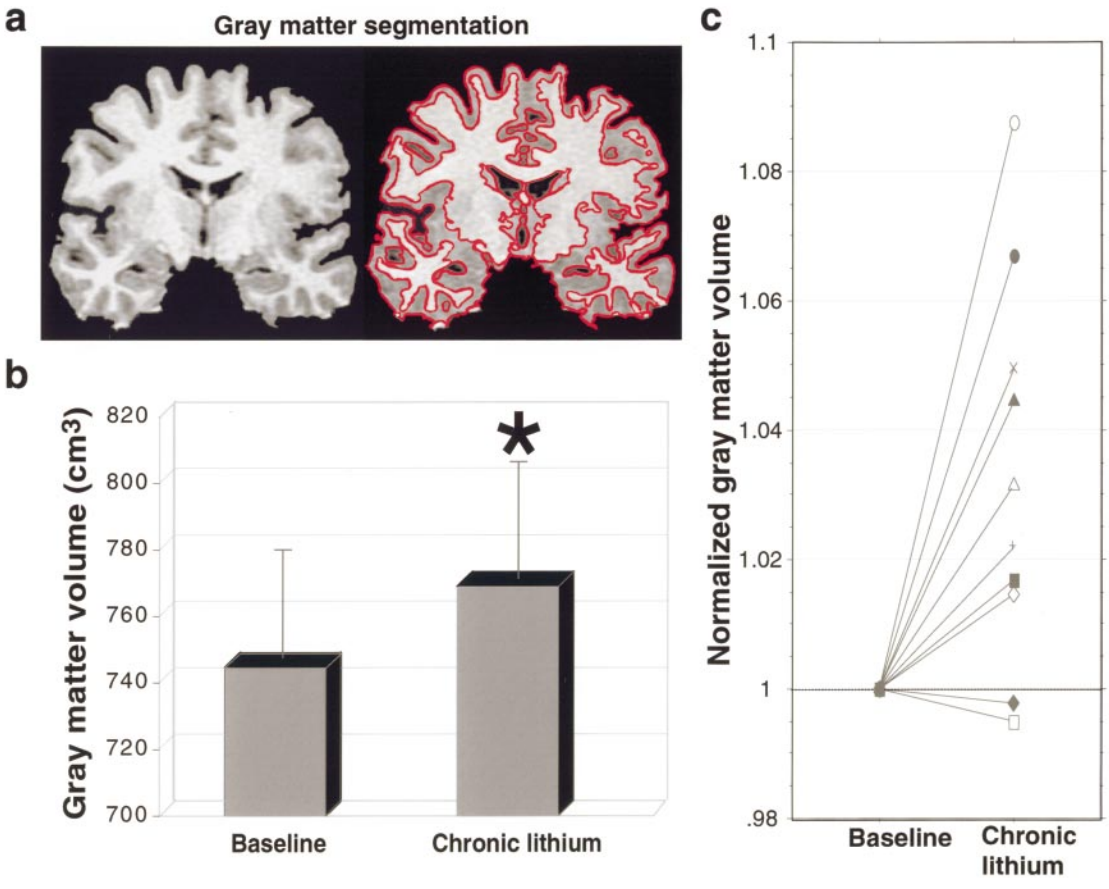

Figure 5 Brain gray matter volume is increased following 4 weeks of lithium administration at therapeutic levels in BPD patients. Inset (a) illustrates a slice of the three-dimensional volumetric MRI data which was segmented by tissue type using quantitative methodology to determine tissue volumes at each scan time point. Brain tissue volumes using high resolution three-dimensional MRI (124 images, 1.5-mm thick Coronal T1 weighted SPGR images) and validated quantitative brain tissue segmentation methodology 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 human brain of patients with BPD. No significant changes were observed in brain white matter volume, or in quantitative measures of regional cerebral water. Modified, and reproduced with permission, from Reference 151.

grained sub-regional analysis of this brain imaging data is ongoing. Since it it believed that the majority of neuron-specific NAA is localized to the neurites rather than the cell body,[152] the observed increase in NAA is likely due to expansion of neuropil content. Such a contention receives additional support from the post-mortem brain study of BPD by Rajkowska and associates,[46] which revealed a significant positive correlation between the relative width of sublayer IIIc and the duration of antemortem lithium treatment. Taken together, these exciting new results support the contention that lithium does indeed exert neurotrophic effects in the human brain *in vivo*.

Concluding remarks

The evidence reviewed in this paper suggests that while MDD and BPD are *clearly* not classical neurodegenerative diseases, they are, in fact, associated with impairments of neuroplasticity and cellular resilience. The cell death and atrophy that has been observed in some patients *despite* the neurotrophic effects of some of our existing psychopharmacologic agents suggests that a reconceptualization about optimal long-term treatment for these disorders may be warranted. We would suggest that somewhat akin to the treatment of conditions like hypertension and diabetes, early and potentially sustained treatment may be necessary to adequately prevent many of the deleterious long-term structural brain changes associated with mood disorders. In this context, it is noteworthy that a recent study of BPD patients showed that while the number of previous episodes was a strong predictor of outcome, it was the first several episodes which accounted for the bulk of the functional decline.[153] Together with the data demonstrating residual deficits in many patients even following a first hospitalization,[154,155] these results highlight the need for early and aggressive intervention, and perhaps even argue for prophylactic treatment in 'high risk' individuals. It is presently

unclear to what extent the cell death and atrophy that occurs in MDD and BPD mood disorders arises due to the magnitude and duration of the biochemical perturbations (eg glucocorticoid elevations), an enhanced vulnerability to the deleterious effects of these perturbations (due to genetic factors and/or early life events), or a combination thereof (Figure 2). While some data suggest that hippocampal atrophy in MDD is related to illness duration,[17] it is presently not clear if the volumetric and cellular changes which have been observed in other brain areas (most notably frontal cortex) are related to the affective episodes *per se*. Indeed, some studies have observed reduced gray matter volumes and enlarged ventricles in mood disorder patients at first onset.[19,156] Furthermore, unlike the situation observed in MDD, MRI hyperintensities in BPD have been found in young patients, and do not appear to be related to cerebovascular disease risk factors. This raises the intriguing possibility that the cell death and atrophy that occurs in BPD may arise more from an endogenous impairment of cellular resiliency, whereas that observed in MDD may be more a manifestation of the neurotoxic sequellae of repeated affective episodes *per se*. A growing body of data is also demonstrating a relationship (potentially bidirectional) between mood disorders and cardiovascular/cerebrovascular disease, suggesting that at least in a subset of patient (? those who have been 'primed' for impairments of cellular resiliency by genetic factors), CNS vascular insufficiency may be a contributory factor.[18,157,158] Clearly the pathogenic mechanisms may be quite distinct in subtypes of mood disorders but preliminary studies have suggested that regional volume brain differences in patients with mood disorders may be associated not only with illness severity/duration, but also with preferential treatment response.[22,159,160]

In conclusion, emerging results from a variety of clinical and preclinical, experimental and naturalistic paradigms suggest that a reconceptualization about the pathophysiology, course and optimal long-term treatment of recurrent mood disorders is warranted. Optimal long-term treatment for these severe illnesses may only be achieved by the early use of agents with neurotrophic/neuroprotective effects, irrespective of the primary, symptomatic treatment. Such treatment modalities, via their effects on critical molecules involved in cell survival and cell death pathways would serve to enhance neuroplasticity and cellular resilience (see Figure 2). Patients who exhibit cell loss and atrophy despite adequate treatment with psychotropic medications known to exert neurotrophic effects, may do so because of potential impairments of the intricate cellular machinery involved in mediating neurotrophic effects (eg CREB/BDNF/trkB/MAP kinase/Bcl-2) at distinct levels. For such patients with putative abnormalities in neurotrophic pathways, improved therapeutics may only be obtained by the more direct targeting of downstream sites. It is thus noteworthy that a variety of strategies to enhance neurotrophic factor signaling are currently under investigation, and the development of selective, CNS-penetrant GSK-3β inhibitors remains an exciting prospect for the future. Furthermore, there is a growing appreciation that mitochondria are critical for regulating cell survival, and that the relative amount of death agonists and antagonists from the Bcl-2 family constitutes a regulatory rheostat whose function is determined, at least in part, by selective protein–protein interactions.[161] An increasing number of strategies are also being investigated to develop small molecule switches for protein–protein interactions, which have the potential to regulate the activity of growth factors, MAP kinases cascades, and interactions between homo- and heterodimers of the bcl-2 family of proteins;[162] these developments hold much promise for the development of novel therapeutics for the long-term treatment of severe mood disorders, and for improving the lives of millions.

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Note added in proof

Jacobs and associates (2000) have recently proposed a novel theory of depression, wherein the waxing and waning of neurogenesis in the hippocampal formation are postulated to represent important causal factors in the precipitation of and recovery from episodes of depression.

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