Neuropathology of Bipolar Disorder

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The literature on the neuropathology of bipolar disorder (BD) is reviewed. Postmortem findings in the areas of pathomorphology, signal transduction, neuropeptides, neurotransmitters, cell adhesion molecules, and synaptic proteins are considered. Decreased glial numbers and density in both BD and major depressive disorder (MDD) have been reported, whereas cortical neuron counts were not different in BD (in Brodmann’s areas [BAs] 9 and 24). In contrast, MDD patients showed reductions in neuronal size and density (BA 9, BA 47). There are a number of findings of alterations in neuropeptides and monoamines in BD brains. Norepinephrine turnover was increased in several cortical regions and thalamus, whereas the serotonin metabolite, 5-hydroxyindoleacetic acid, and the serotonin transporter were reduced in the cortex. Several reports further implicated both cyclic adenosine monophosphate and phosphatidylinositol (PI) cascade abnormalities. G protein concentrations and activity increases were found in the occipital, prefrontal, and temporal cortices in BD. In the PI signal cascade, alterations in PKC activity were found in the prefrontal cortex. In the occipital cortex, PI hydrolysis was decreased. Two isoforms of the neural cell adhesion molecules were increased in the hippocampus of BD, whereas the synaptic protein marker, synaptophysin, was not changed. The findings of glial reduction, excess signal activity, neuropeptide abnormalities, and monoamine alterations suggest distinct imbalances in neurochemical regulation. Possible alterations in pathways involving ascending projections from the brain stem are considered. Larger numbers of BD brains are needed to further refine the conceptual models that have been proposed, and to develop coherent models of the pathophysiology of BD.

Introduction

Prior Reviews of Neuropathology of Bipolar Disorder

Although bipolar disorder (BD) is generally thought to be a brain disorder with a significant genetic component, there have been relatively few neuropathological research studies to date. Advances in molecular biology, which are applicable to postmortem brain studies, make this approach increasingly attractive. The success of this approach requires reasonable hypotheses as where to look and what to look for, questions that may be initially addressed by a review of the neuropathological studies of bipolar disorder. A synthesis of the divergent and sparse literature into a coherent model to generate hypotheses is offered in the present review; however, this review falls short of achieving a full conceptual model, because very limited conclusions can be made at this time from the neuropathological reports.

Scope of the Review

This review is limited to postmortem neuropathology studies of BD reported during 1966–1999. One attractive approach is to organize this review around neuroanatomical structures or circuits. Unfortunately, neither a review of in vivo neuroimaging studies or postmortem studies yields a clear group of structures or circuits implicated in BD. Instead, this review is organized around functional molecular themes as opposed to traditional neuroanatomical regions. These functional themes are related to 1) traditional pathomorphological studies of cell number and architecture; 2) signal transduction pathways; 3) central neurotransmitter systems; 4) neuropeptide and neuromodulatory systems; and 5) specialized subcellular systems that tie these themes together involving the synapse using molecular prototypes as examples. Convergence of these themes is discussed in the final section, with inclusion of more anatomical specificity.

Leads from Illness Manifestations

Are there reasons that one would expect neuropathological examinations to reveal enduring biological changes in brains from BD patients? Or is BD a state phenomenon that does not cause any biological changes observable in the postmortem brain? Neuropathology, which detects
enduring brain changes, may seem less likely to find such state-dependent neurochemical fluctuations. The biogenic amine theory bridging pharmacological observations to mood disorders (Bunney and Davis 1965; Schildkraut 1965) suggests alterations in norepinephrine (NE), and the “permissive hypothesis” integrating serotonin (Prange et al 1974) into mood disorders suggests that neurochemical fluctuations in both of these neurotransmitters contribute to the course of bipolar disease. The fluctuations are difficult to quantitate reliably, because postmortem measurements of neurotransmitters are less stable as compared to receptor and reuptake measurements (Casanova and Kleinman 1990). This leaves the question then of whether work with the postmortem bipolar brain will be useful in detecting changes that occur in brain systems that normally play a role in modulating mood.

Advances in neuroimaging have kindled interest in the pathophysiology of BD. Structural and functional neuroimaging reviews suggest distinct differences in BD in the prefrontal cortex, limbic system, third ventricle, cerebellum, temporal lobe, basal ganglia, and subcortical white matter (Drevets 1998; Soares and Mann 1997). Some differences in neuroimaging studies have been reported in BD; however, many of the findings have not been replicated (for reviews in BD patients see Pearlson 1999; Soares and Mann 1997). Leads obtained from neuroimaging reports can indicate fruitful areas to investigate in postmortem studies. One review of neuroimaging studies (Soares and Mann 1997) concluded that cerebellar changes (DelBello et al 1999; Jeste et al 1988), possible temporal lobe alterations (Altshuler et al 1991; Swayze et al 1992), and increased frequency of subcortical white matter periventricular hyperintensities on magnetic resonance imaging (McDonald et al 1999; Videbech 1997) are representative of findings in BD. Alterations in other brain regions have been recently reported in BD, i.e., reduced gray matter in the left subgenual prefrontal cortex (Drevets et al 1998) and amygdala enlargement in BD (Altshuler et al 1998; Strakowski et al 1999). Clearly, there is a wide range of neuroanatomic structures reported to be abnormal in BD; however, the neuroimaging findings have not suggested specific cellular substrates in these regions and it is expected that further refinements from neuroimaging will be forthcoming when magnetic resonance imaging reports derived from specific neurochemical markers such as γ-aminobutyric acid and N-acetylaspartate are published.

A compelling reason to examine the postmortem brain is that it offers finer resolution at the cellular and molecular level than either current functional or structural neuroimaging techniques which are limited to millimeter-level resolution. This resolution and connections within the brain currently offers the best opportunity to invoke biological explanations and determine the pathophysiology of bipolar illnesses.

**Cellular Pathomorphology**

**Cortex**

One of the most thorough postmortem investigations of BD cellular distribution is the study by Ongur et al (1998). These investigators examined one of the largest number of bipolar cases ($n = 18$) from both the Stanley Consortium ($n = 14$) and Harvard Brain ($n = 4$) collections using stereological assessments of neuronal and glial numbers in area 24 of the ventral prefrontal cortex. Glia were defined simply as small round nuclei seen in Nissl-stained sections. Both BD and major depressive disorder (MDD) brains were examined and divided into “familial” and “other” cases. There were significant reductions in glial density in the subgenual prefrontal cortex for BD and MDD found in both the Stanley and Harvard brain collections. The glial density reductions were examined by subgroup in the Stanley collection, and significant reductions were found in familial cases for BD ($n = 4$) and MDD ($n = 6$; Ongur et al 1998). The “other” cases for BD ($n = 10$) and MDD ($n = 3$) showed a normal glial density. Neuronal number and density were unchanged in BD and MDD (Ongur et al 1998). In the somatosensory cortex, there were no significant differences in neuronal and glial number between the groups (Ongur et al 1998). The number of glia with nuclear volumes of 75–150 μm$^3$ were significantly reduced in the familial BD group compared with control subjectss (Ongur et al 1998). Although the bipolar and schizophrenia patients’ brains had a significantly longer time in fixative than the control subjectss, the patients with schizophrenia had normal glial density. Is the neuropathology related to mood disorder in general, and if so to which glia subtypes? This study does not specifically address whether the changes are specific to astrocytes, microglia, or oligodendroglia. Astrocytes are the most likely candidate, but further studies would be needed to define the glial subtype that is involved as well as the implications of a 50% reduction in total glial numbers.

There have been several additional studies that have examined neuronal and glial number in BD and MDD. Neuronal density and laminar neuronal density were normal in the dorsolateral prefrontal cortex (i.e., Brodmann’s area [BA] 9 of bipolar brains, whereas a 6% decrease in cortical thickness was noted (Rajkowska 1997)). The author suggests that the reduction in cortical thickness may reflect a reduced number of neurons present in the cortex; however, glial density was not evaluated and is equally likely to have contributed to the reduction in cortical thickness.
Although no bipolar patients were examined, a detailed study of cell measurements in MDD \((n = 12)\) in three frontal regions (Rajkowska et al 1999) sheds light on abnormalities reported earlier in both bipolar and depressive disorders (op cit). Decreased cortical thickness was found in the rostral orbitofrontal (BAs 10–47) and middle orbitofrontal cortex (BA 47\(^2\)), whereas caudal orbitofrontal (47\(^2\)) and dorsolateral PFC (dIPFC, BA 9) were normal (Rajkowska et al 1999). Mean neuronal cell body size was reduced in the rostral orbitofrontal cortex. The laminar analysis of glial density showed a significant reduction in medium and large glial cells in layers IIIa and IV in MDD patients. The caudal orbitofrontal cortex, although not significantly thinned, showed a reduction in mean neuronal size in layer II, and lower density of large neurons in layers IIIa and IV. Striking reductions in overall glial cell densities were widespread and evident in the layers IIIc–VI of caudal orbitofrontal cortex. In the dIPFC, cortical thickness was similar between control subjectss and MDD; however, the mean neuronal cell body size was decreased in layers III and VI. The density of large neurons was reduced in layers II, III, and VI of the PFC. The mean glial density was reduced in layers III and V of the dIPFC in MDD. The authors suggest a rostral–caudal gradient of changes in the PFC in MDD and the most changes occurring in layers II–III, which are among the last layers to completely develop after birth. Although an effect of antidepressant medications on the pathologic changes observed has not been ruled out, two patients that had never been treated with antidepressant medication also showed similar changes (Rajkowska et al 1999). These results suggest that gray matter thinning in MDD might be related to both the reductions in neuronal size and glial number.

Although determining which glia are responsible for the changes in the Ongur et al (1998) and Rajkowska et al (1999) studies has not been determined, study of the glial cell density in the corpus callosum found no difference between control subjectss and BD (Nasrallah et al 1983). Because the corpus callosum contains mainly oligodendrocytes, these data restrict the possibility that changes in glial numbers in PFC gray matter are more likely to involve astrocytes and microglia than oligodendrocytes.

The findings of both Ongur et al (1998) and Rajkowska et al (1999) demonstrate reductions in glial number in the cortex as the most prominent pathophysiological feature of BD and MDD. The possible circumstances that could result in large reductions in glial numbers and the impact of such changes on neuronal function are of interest. Because the changes in glial numbers were found in certain cortical regions only, a generalized abnormality in glial development or function is unlikely to be responsible. It is more probable that the changes are secondary to connectivity with other brain regions and alterations in the function of neural circuits (Figure 1). Conversely, a change in glial number is likely to have effects on the function of local neurons. Alterations in ion homeostasis, neurotransmitter uptake and release, and production and release of neurotrophic factors have been associated with glial functions. Certainly, a large alteration in glial number in the cortex would be expected to have a marked effect and may be an important contributor to the pathophysiology of BD.

**Limbic System**

Nonpyramidal (NP) neuronal number and densities were significantly decreased in the CA2 sector of the hippocampus of patients with both BD \((n = 4)\) and schizophrenia \((n = 10)\) compared to control subjects \((n = 11)\; \text{(Benes et al 1998)}\). Moreover, there were no other differences in the pyramidal neurons or NP neurons throughout the CA1, CA3, and CA4 sectors between any groups. Although medication effects cannot be excluded, in each group one drug-free patient also showed lowered NP cell counts. This is a preliminary study of BD and neuron counts in the hippocampus and the interpretation is premature owing to the small sample size of BD patients; however, these findings implicate the possible loss of inhibitory control exerted by the NP \(\gamma\)-aminobutyric acid (GABA)-ergic interneurons over pyramidal cells in the hippocampus (Svoboda et al 1999).

Entorhinal cortex (ERC) malformations were found in the architecture of the clusters of medium- to large-size polygonal stellate nerve cells (layer II island) patients with BD \((n = 4)\) compared to control subjects (Beckmann and

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**Figure 1.** The neuroanatomic circuits that are potentially involved in bipolar disorder (BD). The potential alterations in neurotransmitter systems in the brain stem and projections to other brain regions are shown. The circuit diagram is adapted from prior suggestions (Baumann et al 1999b; Ressler and Nemeroff 1999; Soares and Mann 1997). The permissive hypothesis suggesting alterations in both serotonin (5-HT) and norepinephrine (NE) is adapted from Prange et al 1974. Postmortem studies of BD have generally shown alterations in the cortex, hippocampus, locus coeruleus (LC), and hypothalamus. n., nucleus.
The malformed architecture was observed in the whole rostral entorhinal area laterally up to the perirhinal area and to the temporal isocortex. In one case, the characteristic islands of layer II of the ERC were completely missing. These preliminary observations of abnormalities are suggestive of a disturbance of young neurons migrating in the deeper layer; however, this disturbance does not appear to be specific for BD, as patients with schizophrenia also show an apparent displacement of layer II-type neurons deep into layer III and other cytoarchitectonic disturbances (Arnold et al 1991), which was not subsequently confirmed at least in schizophrenic patients (Bernstein et al 1998; Krimer et al 1997).

Nucleolar size may reflect the nuclear health of a cell, and reductions in nucleolar size are associated with neurodegeneration. Insofar as nucleolar size of ERC stellate cells (layer II) were found to be normal in leukotomized bipolar patients (n = 5) compared to nonleukotomized control subjects (Casanova et al 1992), neuronal degeneration is not thought to be related to hypothesized abnormalities in the second layer of the ERC.

Subcortical

Neuroimaging studies have implicated basal ganglia structures in mood disorders. For example, MDD patients show smaller basal ganglia volumes, whereas BD patients show a normal basal ganglia volume, and according to some studies there is an increased caudate volume in BD (Soares and Mann 1997). A postmortem study found smaller basal ganglia volumes in MDD (n = 4) compared to control subjects (Baumann et al 1999a), most notably there were reductions in the external pallidum, putamen, and nucleus accumbens. This study included only two BD patients and did not report data separately for the BD patients, so that any generalization to BD is limited.

One of the major sources of NE within the central nervous system (CNS) is large pigmented neurons in the locus coeruleus (LC) that contain melanin (Ordway 1997). These neurons project to multiple cortical (layers I, IV, and V, primarily) and subcortical areas (hypothalamus and thalamus). The LC neurons can send collateral branches to the neocortex, hippocampus, cerebellum, and spinal cord simultaneously (Green et al 1989). There is evidence of a limited topographical organization of the LC in projections to the cortex (for review of NE and mood disorders see Ressler and Nemeroff 1999). The total pigmented and the total large pigmented LC neuronal counts of BD patients (n = 6) was increased bilaterally as compared to MDD patients (n = 6; Baumann et al 1999b). The increased number of pigmented neurons was more apparent in the rostral two-thirds and dorsal part of the LC. Although there were more pigmented neurons counted in BD patients than control subjects (n = 12), this difference did not reach statistical significance (Baumann et al 1999b). In other studies of LC neuronal counts, MDD patients were normal (Klimek et al 1997), whereas suicide victims and alcoholics showed reductions in pigmented LC neuron counts (Arango et al 1994, 1996). Although the data at this time support a trend for an increased number of LC neurons in BD compared to control subjects, a simplistic interpretation of the increased number of LC neurons in BD compared to MDD is that more NE is produced and possibly released bilaterally in the cortex of patients with BD. The relation of the increased numbers of LC neurons and the effect on possible down-regulation of glial cell numbers would be an interesting correlative study between brain regions. Further research into whether tyrosine hydroxylase, the vesicular monoamine transporter, NE transporter, and β-adrenergic receptor levels are altered in the LC and the LC projection sites, such as the frontal cortex, are needed to determine whether a dysregulation of NE in patients with BD does exist. Thus, further postmortem analyses are required in cohorts of patients where both the LC and the multiple projection sites of the LC are analyzed together to determine the pathways of dysregulation of NE in BD.

Cerebellar Structural Abnormalities

Abnormalities have been reported in the cerebellum for BD (Loeber et al 1999), although the neuroimaging evidence to support abnormalities is not unequivocal (Nasrallah et al 1989; Soares and Mann 1997). The pathophysiology of possible cerebellar vermis abnormalities in BD (n = 12), MDD (n = 15), and control subjects (n = 15) was investigated (Helmkamp et al 1999). Displaced Purkinje-like cells were counted in the superior cerebellar vermis and evaluated as displaced if 1) displacement into the intrafoliar white matter or the adjacent internal granular layer was observed; and 2) the cell bodies were at least 30 μm in size. There were no significant differences in displaced Purkinje-like cells in the superior vermis of patients with BD or MDD groups compared to control subjects.

Signal Transduction—Neurochemical Pathology

The biogenic amine theory (Bunney and Davis 1965; Schildkraut 1965) is based upon a bridge between pharmacological alterations of monoamines and modulation of affective disorders. Depletion of amine neurotransmitters leads to an increased incidence of affective disorder, as shown by the administration of the antihypertensive drug reserpine, a drug that depletes aminergic neurotransmitters...
(norepinephrine, serotonin, and dopamine). Conversely, evidence is derived from observations that tricyclic antidepressants are thought to exert their effects by increasing norepinephrine and serotonin levels at the synapse by competition with their respective transporters. The increase in neurotransmitters, e.g., norepinephrine, could act via the cyclic adenosine monophosphate (cAMP) and phosphoinositol second messenger signal cascade systems (Table 1). Other systems have also been implicated in the pathophysiology of BD, such as disturbances in sodium- and calcium-transport but have been examined to a lesser degree.

**G-Coupled Proteins and Cyclic AMP System**

G proteins couple both to cell surface receptors and to effector proteins (e.g., enzymes such as adenyl cyclase). G proteins are heterotrimeric and utilize guanosine triphosphate (GTP) as a cofactor in coupling effector to receptor proteins. Receptor activated G proteins dissociate typically into α and β/γ subunits, with approximately 20 different α, five β, and 12 γ subunits described (Spleiss et al 1998). G αs stimulates adenyl cyclase, whereas G αi inhibits adenyl cyclase. Activation of adenyl cyclase leads to an increase of cAMP second messenger. G proteins are components of the intracellular signal cascade for multiple receptor systems (Table 1) including dopamine receptors (D1, D2); adrenergic receptors α2, β1, and β2; serotonin receptor, 5-HT1; histamine receptor, H2; purine receptors, A1 and A2; amino acid receptor, GABA; and multiple peptide systems, such as the vasopressin, V2 receptor, and μ- and δ-opioid receptors (Grebb and Browning 1989). Since the seminal works (Dousa and Hechter 1970; Forn and Valdecasas 1971) showing that lithium inhibits adenyl cyclase activity in brain, albeit in dosages above the therapeutic dosage for BD, published reports of adenyl cyclase activity or G proteins in patients with BD began to appear. There have been a number of studies looking at changes of G proteins in postmortem BD patients. Although most studies are confounded by drug treatment effects, the evidence suggests an involvement of G-coupled protein pathways in BD.

**G αs**

The G αs subunit has been measured in leukocytes and neutrophils of bipolar patients. G αs protein and mRNA were increased in lithium-treated and nonmedicated patients, as compared to control subjects or MDD (Spleiss et al 1998; Young et al 1994a). Further, G αs subunit was also increased in lithium-treated bipolar patients but not unmedicated bipolar patients (Spleiss et al 1998).

The first postmortem studies on G αs subunits (45 kd and 52 kd) in occipital cortex and prefrontal cortex (52 kd) reported elevations in BD (n = 7), although the changes in cerebellum were not significant (Young et al 1991). A second study showed increased G αs in frontal (36%), temporal (65%), and occipital (96%) regions in BD (n = 10) compared to control subjects (Young et al 1993). In contrast to the neocortex, other regions that did not show any difference in G αs included the thalamus, hippocampus, and cerebellum. There were no differences in G αs for any brain region measured in patients with schizophrenia or Alzheimer’s disease. No differences were found for the other G protein subunits, G αi, and G β in all regions examined. In BD cases there was increased forskolin-stimulated adenyl cyclase activity in temporal and occipital cortex, but not in frontal cortex (Young et al 1993). In both G αs protein studies (Young et al 1993; Young et al 1991) the same seven BD and matched control pairs were measured. The increased cAMP formation and G αs protein suggests increased adenyl cyclase activity in BD, although this increased activity might also be related to an increase in adenyl cyclase concentration. There

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**Table 1. The Major Neurotransmitter Receptor Families in the Central Nervous System Activate Different Signal Pathways**

<table>
<thead>
<tr>
<th>G protein–coupled receptor</th>
<th>cAMP</th>
<th>Phosphoinoside</th>
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<tbody>
<tr>
<td>Acetylcholine</td>
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<td>+</td>
</tr>
<tr>
<td>Muscarinic</td>
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<td>+</td>
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<tr>
<td>Amino acid</td>
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<td>+</td>
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<tr>
<td>γ-Aminobutyric acid</td>
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<td>+</td>
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<tr>
<td>Glutamate–metabotropic</td>
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<td>–</td>
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<tr>
<td>Type I</td>
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<td>+</td>
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<td>Type II</td>
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<td>Type III</td>
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<tr>
<td>Dopamine</td>
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<td>D1</td>
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<td>D2</td>
<td></td>
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<td>Histamine</td>
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<tr>
<td>H1</td>
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<tr>
<td>H2</td>
<td></td>
<td>–</td>
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<tr>
<td>Norepinephrine</td>
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<tr>
<td>α1</td>
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<td>β1</td>
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<td>β2</td>
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<tr>
<td>Peptide</td>
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<td>–</td>
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<tr>
<td>Opioid</td>
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<tr>
<td>δ</td>
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<tr>
<td>µ</td>
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<tr>
<td>Vasopressin</td>
<td></td>
<td>+</td>
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<tr>
<td>V1</td>
<td></td>
<td>+</td>
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<tr>
<td>V2</td>
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<td>+</td>
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<tr>
<td>Serotonin</td>
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<td>–</td>
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<tr>
<td>5-HT1</td>
<td></td>
<td>+</td>
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<tr>
<td>5-HT2</td>
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<td>+</td>
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</tbody>
</table>

*Activation (+) or inhibition (−) of second messenger system by neurotransmitter. This table is not meant to be exhaustive of all neurotransmitters but is meant to convey the idea that neurotransmitters can activate both second messenger pathways. cAMP, cyclic adenosine monophosphate. (Adapted from Snider et al (1987) and Grebb and Browning (1989).*
are multiple adenyl cyclase isofoms in brain illustrating the complexity of testing second messenger pathways in postmortem analysis of BD. The significant results that were found only in the cerebral cortex suggest a widespread, yet variable, receptor-coupled pathophysiology in brain.

A systematic test of specific G α proteins that might be associated with the pathophysiology of BD was conducted by examining five G α subunits (s, i, o, z, and q) for both protein concentration and activation (Friedman and Wang 1996). All BD brain tissue (n = 5) showed no detectable lithium levels (<0.05 mEq/L). There was an overall increase of 34–48% in binding of guanosine 5′-O-3-thiophosphate (GTP) to G α proteins in BD prefrontal cortex. Agonist stimulated (adrenergic, muscarinic, and 5-HT) increases in GTP binding to the G α proteins were noted except for G αs. Individual levels of G α proteins were increased in BD prefrontal cortical membranes by G αs 51 kd (56%) and G αo 45 kd (38%), whereas the G αi, G αo, G αz, and G αq were the same in bipolar and control subjects (Friedman and Wang 1996). Further, the heterotrimeric state of G proteins, detected by an assay of pertussis-activated adenosine diphosphate ribosylation, was increased in the frontal cortical membranes of BD patients. It is still possible that the data might represent lasting effects of lithium on G proteins following lithium treatment (Lenox et al., 1998); however, the mechanism by which enhanced agonist induced G protein GTP binding occurs in BD is not known.

The evidence for alteration in G αs protein and mRNA found in studies of bipolar patients’ leukocytes and neutrophils (Spleiss et al. 1998; Young et al. 1994a) was initially replicated in two studies of G αs protein in cerebral cortex (Friedman and Wang 1996; Young et al. 1993), whereas a third study failed to show any difference in G αs protein (Dowlatshahi et al. 1999) in BD (n = 15) using the Stanley Consortium samples. A study of G αs mRNA levels in three cortical regions in BD (n = 10) also showed no differences (Young et al. 1996). No firm conclusions concerning relative importance of G αs protein in BD can be made, as the same laboratory has not consistently replicated differences in the cortex. In general these studies used samples of BD and control subjects that were matched for postmortem interval, age, and gender. Differences in patient populations, drug treatment state, sample properties from various brain banks, and other variables inherent to postmortem biochemical studies are likely to contribute to these variations. The G αs protein heterotrimeric state rather than the concentration might be a critical factor.

Patients with MDD did not show alterations in G αs in the parietal and temporal cortices, whereas alcoholics showed a reduction in G αs (52 kd) in temporal cortex as compared to control subjects (Ozawa et al. 1993a, 1993b). Chronic lithium did not alter levels of G protein subunits (G αs, G αo, and G β), whereas decreased levels of G αi, and G αz were found for the inhibitory subunits (Colin et al. 1991). These studies indicate that the potential upregulation of G αs in BD is not likely to be due to depression, lithium treatment, or alcohol.

G protein α subunits were examined in depressed suicide (Pacheco et al. 1996) in BAs 8/9 and BA 10 frontal cortex. In BA 10 there was a 68% increase in G αs (45 kd), and in BAs 8/9 there was a significant decrease (21%) in the level of G αs in the depressed suicide group. Levels of other G α subunits (q11, i1, and o1) were not different in depressed suicide in either brain region. Depressed patients showed decreases in G αs (Ozawa et al. 1993a, 1993b), whereas depressed suicide patients had increased G αs (Pacheco et al. 1996). The range of results showing alterations in G αs levels and activity further indicate that G-coupled proteins may fluctuate with chronic drug treatment, postmortem interval, and a host of other factors not associated with mood state and BD per se.

Other G Proteins

G αq11 protein was increased 62% in occipital cortex from BD (Mathews et al. 1997). Phospholipase-C (PLC)-β1 protein was also increased (52%) in the occipital cortex of the bipolar subjects (n = 10), but only reached marginal statistical significance (p = .07). Frontal and temporal cortex G αq11 or PLC-β1 protein concentrations were not different between BD and control subjects. Cerebral cortical protein levels of G β1 or G β2, included as a negative control, were not different between groups (Mathews et al. 1997). The pathophysiological meaning of the regional and subunit differences in cortex second messenger pathways mediated with G-coupled proteins is unclear.

CAMP-Dependent Protein Kinase

cAMP quickly degrades in postmortem brain and thus cannot be measured directly. As an alternative, binding of [3H]cAMP to the cAMP-dependent protein kinase has been utilized as an indirect measure of cAMP (Rahman et al. 1997). [3H]cAMP dependent protein kinase (cAMP-dPK) binding was measured in membrane and cytosolic fractions from BD. Bipolar patients (n = 10) showed significant reductions in binding in the cytosol across frontal (−22%), temporal (−23%), occipital (−22%), and parietal (−15%) cortex, in the cerebellum (−36%), and in thalamus (−13%), but not in membrane fractions (Rahman et al. 1997). Lithium administration increases binding of cAMP to cAMP-dPK in rat brain (Mori et al. 1998); therefore, the decreases observed in BD (Rahman et al. 1997) may not be due to the effect of chronic lithium on cAMP-dPK binding activity.
Maximal cAMP-dPK enzyme activity was significantly higher (104%) in temporal cortex cytosolic fractions from BD brain (n = 10) as compared to matched control subjects (Fields et al 1999). Basal cAMP-dPK activity was also significantly higher (40%) in temporal cortex cytosolic fractions of BD brain compared with control subjects. Estimated EC50 values for cyclic AMP activation of cAMP-dPK were significantly lower (70% and 58%, respectively) in both cytosolic and membrane fractions of temporal cortex in BD (Fields et al 1999).

Phosphoinositide-Mediated Pathway

The phosphoinositide signal transduction system is a second messenger system that is activated by multiple CNS receptor systems (Table 1), some that are coupled to G proteins (Snider et al 1987). Intensively studied examples are the α, β-adrenergic, serotonergic 5-HT2, and muscarinic cholinergic receptors. The phosphatidylinositol (PI) pathway generates two important second messenger signals from metabolism of phospholipids in the membrane, inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG). IP3 is inactivated by a phosphohydrolase to IP2 (inositol biphosphate), which is further converted to inositol monophosphate (IP). Inositol monophosphate is converted by inositol monophosphate phosphatase to inositol (myo-inositol) and the inositol is then available for re-synthesis of PI. One action of lithium is to block the conversion of IP to inositol by interfering with inositol monophosphate phosphatase conversion of IP to inositol. This “lithium effect” (Sherman et al 1985) would result in decreased inositol in brain tissue and a prediction of increased myo-inositol-1-phosphatase activity (Bunney and Garland-Bunney 1987).

In a direct test of the “lithium effect” hypothesis, free inositol levels in postmortem brain from BD (n = 8) and suicide victims (n = 10) were compared (Shimon et al 1997). PFC levels of inositol in BD were lower than control subjects, but inositol was also lower in suicide victims when two subjects with postmortem intervals of >30 hours were excluded. There was detectable lithium in three of the BD brains and no detectable lithium in the remaining five patients. There was no significant difference for free inositol in the occipital cortex or cerebellum, although there was a trend noted in the occipital cortex to be somewhat lower in both BD and suicide victims. There was no difference in inositol monophosphate activity between BD, control subjects, and suicide victims (Shimon et al 1997). Insofar as inositol levels and inositol monophosphate activity do not decline significantly during the first 24 hours postmortem period in rat brain (Belmaker et al 1998), the study may not be confounded by postmortem degradation. In rats, lithium treatment does not dramatically alter inositol levels in the cerebellum, cortex, caudate, or hippocampus, but does lead to a significant decrease in the hypothalamus, a brain region that could be involved in mood regulation (Belmaker et al 1998). In contrast, an in vivo magnetic resonance spectroscopy study of inositol depletion in BD patients (n = 12) showed that lithium treatment does reduce inositol levels in the right frontal cortex (Moore et al 1999). Reductions of inositol, a precursor in the PI signal pathway, can affect the downstream intraneuronal signal cascade, such as by interactions of IP3 and DAG with Ca2+ release and protein kinase C (PKC) activity; however, the reduction in inositol appears much sooner than improvement in clinical state (Moore et al 1999), thereby implying that other pathways are involved in long-term effects of lithium-induced inositol reduction in BD. These pathways would be mediated by PKC activity, which can trigger multiple changes in protein phosphorylation and gene expression.

The G protein–coupled phosphoinositide signal system was shown to be impaired in BD brain tissue (n = 10) from occipital cortex, but not frontal or temporal cortex (Jope et al 1996). The degree of impairment in hydrolysis of PI correlated with brain lithium concentrations in the occipital cortex. Further, phospholipase C activity was not different in membrane preparations. Thus, the components of the phosphoinositide system appear to be regionally variable in postmortem human brain (Pacheco and Jope 1996).

Protein Kinase C

Protein kinase C (PKC) translocates from the cytosol to membranes, and lithium has been implicated in inhibiting PKC translocation (Wang and Friedman 1989). Specific PKC membrane-associated activity was significantly higher in BD patients (n = 5) frontal cortex (Wang and Friedman 1996). Specific concentrations of PKC isozymes were elevated (cytosolic α- and membrane-associated γ and ζ PKC isozymes) and cytosolic epsilon PKC was decreased. Cytosolic PKC enzyme activity stimulated with phorbol 12-myristate 13-acetate (PMA) was decreased in BD (Wang and Friedman 1996); however, the translocation of PKC activity from cytosol to membrane by PMA was increased in brain slices from BD subjects. Further, lithium ion was not detectable in any tissue examined. Differentially regulated PKC isozymes in BD brain could affect phosphorylation of downstream third messenger proteins.

Calcium and Sodium

Na,K-ATPase Hypothesis

The hypothesis that the sodium ion pump or Na,K-adenosine triphosphatase (Na,K-ATPase) is altered in BD
(Singh 1970) was put forth to account for electrolyte and fluid imbalances observed in mood disorders. Meta-analysis of erythrocyte studies of the sodium pump indicated that there is a modest reduction of erythrocyte Na,K-ATPase activity levels in bipolar patients (Looney and el-Mallakh 1997). Significant reductions of Na,K-ATPase activity in bipolar patients compared to euthymic bipolar patients (effect size = −.42) and bipolar depressed compared to bipolar euthymic (effect size = −.62) were noted (Looney and el-Mallakh 1997).

The level of Na,K-ATPase protein (α 2 subunit) was reduced in the temporal cortex of BD patients (n = 5), whereas normal levels of the Na,K-ATPase α 1 and 3 subunits were found (Rose et al 1998). Na,K-ATPase α 3 subunit is restricted to neuronal expression, whereas the α 2 subunit isoform is more diffusely spread throughout the CNS in glia, predominantly astrocytes (Cameron et al 1994). The reduction in the glial isoenzyme of the Na,K-ATPase protein (α 2 subunit) agrees with research showing fewer glial cells in the frontal cortex (Ongur et al 1998). The Na,K-ATPase hypothesis was weakly tested by linkage of a dinucleotide polymorphism near the Na,K-ATPase α 3 subunit gene in a BD association study (Mynett-Johnson et al 1998). The likelihood of a functional change in expression of this gene is unknown, because the polymorphism has not been conclusively demonstrated to be within the Na,K-ATPase α 3 subunit gene.

Catecholamine and Indoleamine Systems

It has been suggested that a reduction in both catecholamine and indoleamine levels may predispose to an affective disorder, with depression resulting from decreased catecholamine and mania being the result of increased catecholamine increases (Bunney and Davis 1965; Prange et al 1974; Schildkraut 1965). A few postmortem studies involving bipolar patients have formally examined either catecholamine or indoleamine pathways; however, there has not been a formal test of the permissive hypothesis conducted in postmortem studies, i.e., an examination of 5-HT and NE levels in the same BD brain samples.

The levels of NE, serotonin (5-HT), dopamine (DA) were not different in BD brain (n = 9) compared with control subjects (Young et al 1994c). Norepinephrine turnover was significantly increased in BD in frontal-, temporal-, occipital-cortex, and thalamus (64–107%). Significant decreases were found in the major 5-HT metabolite, 5-hydroxyindolacetic acid (5-HIAA), in frontal (−54%) and parietal cortex (−64%). In addition, levels of the major DA metabolite, homovanillic acid (HVA) were significantly decreased (−46%) in parietal cortex and HVA/DA ratios were significantly reduced (−66%) in occipital cortex obtained from BD compared to control subjectss (Young et al 1994c). The significant increase in NE turnover and decreased 5-HT metabolite concentration imply an alteration in the balance of NE and 5-HT. If an increase in NE turnover arises from increased NE, the imbalance between NE and 5-HT would be consistent with the permissive hypothesis of BD. Because monoamines and their metabolites are rather unstable postmortem, a number of investigators have focused their efforts on the receptor and reuptake sites.

Although the serotonin transporter has been studied extensively in MDD and suicidal patients, there has been only one study of BD patients (Leake et al 1991). Serotonin uptake sites have been measured, using the high-affinity selective serotonin reuptake inhibitor 3H-citalopram, in postmortem frontal cortex (BA 9) from BD (n = 6), MDD (n = 9), and matched control subjects (Leake et al 1991). A significantly lower concentration of 3H-citalopram binding in frontal cortex was found in BD (60% of control binding) and MDD (78% of control binding). Although the effect of antidepressant treatment can not be ruled out, there were no differences in 3H-citalopram binding for the combined mood disorder patient group (BD and MDD) who were free of antidepressant medication (mean = 37 months) as compared to those mood disorder patients not taking antidepressants. A reduction in 5-HT transporters can be considered as consistent with a reduction in synaptic 5-HT, according to data showing that the 5-HT transporter is downregulated following chronic depletion of 5-HT by administration of p-chlorophenylalanine methyl ester (Linnet et al 1995). If a reduction in the 5-HT transporter is indicative of a reduced level of 5-HT function, then the NE and 5-HT data mentioned above are consistent with the permissive hypothesis of alterations in both neurotransmitters in BD (Mann et al 1986; Prange et al 1974); however, it can also be considered that a reduction of 5-HT transporter would lead to an increased 5-HT at the synapse, and this interpretation would not support the permissive hypothesis.

Similarly, although 5-HT and NE receptors have been studied extensively in MDD and suicidal patients, there is only one study in BD (Young et al 1994b). Cerebral cortex β-adrenergic receptor binding, measured by [125I]-iodopindol binding, in frontal, occipital, or temporal cortex in BD was not different than control subjects (Young et al 1994b). The binding affinity for [125I]-iodopindolol was normal in BD occipital cortex, the only cortical region measured. These results do not support alterations in the density or affinity of β-adrenergic receptors in cerebral cortex in BD in a tissue homogenate; however, the distribution of the β-adrenergic receptor subtypes in the
Neuropeptides, Neuromodulators, and Neurotrophins

The hypothalamic–pituitary–thyroid (HPT) and/or hypothalamic–pituitary–adrenocortical (HPA) brain systems are fundamental to the neuroendocrine adaptive stress responses and perhaps to the underlying pathophysiology of mood disorders. BD patients have an increased frequency of disturbances in the HPT (Kirilke et al. 1988) and/or HPA axes (Rush et al. 1996). The role of stress on glucocorticoid output has been examined for several decades. Further, hypercortisolemia in BD patients (Zisook et al. 1985) and dexamethasone nonsuppression (Rush et al. 1996) could affect the cortex via glucocorticoid receptors found throughout the cortex (Seckl et al. 1993). Chronic hypercortisolemia is also toxic to hippocampal neurons (Brown et al. 1999; Kiraly et al. 1997), promotes hippocampal dysregulation (Young and Vazquez 1996), and disrupts neurogenesis in the hippocampus (Eriksson et al. 1998; Gould and Tanapat 1999). Disruption of neurogenesis in the dentate granule cells of the hippocampus does not occur via direct cortisol receptor stimulation, as there is an absence of Type 1 or Type 2 adrenal steroid receptors in the granule cell precursors (Gould and Tanapat 1999). Instead, the activation of N-methyl-D-aspartate (NMDA) receptors in the perforant pathway appears to be the downstream component of cortisol stimulation (Cameron et al. 1998). Thus, one consequence of hypercortisolemia would be a down-regulation of neurogenesis in the granule cells of the hippocampus of patients with BD.

Neuropeptides–Neuromodulators

Early reports suggested alterations in vasopressin (AVP; Gold and Goodwin 1978), somatostatin (Gerner and Yamada 1982), endorphins (Verebey et al. 1978), and other neuropeptides in BD. A few studies of postmortem BD brain and neuropeptides have since followed.

Increased AVP- and oxytocin (OXT)-expressing neurons in the paraventricular nucleus (PVN) of the hypothalamus were found in MDD \( n = 3 \) and BD \( n = 3 \) combined group (Purba et al. 1996). The numbers of AVP- and OXT-immunoreactive neurons in the PVN of patients with mood disorder were increased by 56% and 23%, respectively. No differences were found in AVP- or OXT-cell numbers between the MDD patients and BD patients. An increased number of corticotropin-releasing hormone (CRH) expressing neurons (four-fold greater than control subjects) was found in the hypothalamic PVN of the combined BD and MDD group (Raadsheer et al. 1994). The colocalization of AVP and CRH in neurons was almost three times higher in the combined MDD and BD group as compared to the control group. This study potentially demonstrates the hyperactivity of CRH neurons, which could be directly related to activation of the HPA axis in BD and MDD subjects.

Neuropeptide Y (NPY) mRNA expression levels in the prefrontal cortex (BAs 9 and 46) was reduced in the group of BD subjects \( n = 15 \) as compared to the control subjects (Caberlotto and Hurd 1999). Neuropeptide Y mRNA expression in MDD and schizophrenia groups were normal. A separate study found that NPY protein expression in frontal cortex was not different in MDD, alcohol dependence, and control subjects (Ordway et al. 1995). Thus, the finding that neuropeptide Y mRNA is reduced in the prefrontal cortex of BD appears to be disease specific.

Although the total number of BD subjects examined is small, the alterations in NPY, and OXT, AVP, and CRH, which regulate the HPA axis, offers preliminary but intriguing evidence to support further investigations of alterations in neuropeptides in affective illness.

Neurotrophins

A number of growth factors, cytokines, and chemokines have been shown to influence glial and neuronal density in the brain (e.g., Davies 1996). Candidate genes that regulate CNS development have not been extensively explored with the exception of one study in BD (Schramm et al. 1998). Tyrosine kinase (Trk) C receptor mRNA in the prefrontal cortex, temporal cortex, and cerebellum for BD was not different compared to control subjects (Schramm et al. 1998). A 5.8-fold reduction of trk C mRNA in the frontal cortex was, however, found in schizophrenia. Thus, further work is clearly needed to determine the involvement of neurotrophic factors in BD. Along these lines, a suggestion has been made that part of the therapeutic effects of lithium might involve neurotrophic-like effects (Manji et al. 2000).

Cell Adhesion Molecules and Synaptic Markers

Cell Adhesion Molecules

Cell adhesion molecules (CAMs), which include the neural cell adhesion molecule (N-CAM) and L1, are candidate molecules that might be involved in causation or disease process in neuropsychiatric disorders. Cell adhe-
sion molecules are involved in synaptic development and plasticity, and in development of the CNS. Neural cell adhesion molecules and L1 play important roles in many neurodevelopmental processes, including axonal guidance, synapse stabilization, hippocampal long-term potentiation, and cell migration (Cunningham et al 1987; Luthi et al 1996; Martin and Kandel 1996; Ronn et al 1998). Early studies did not evaluate the individual CAM isoforms. Recently, measurement of CAM and synaptic proteins in the cerebrospinal fluid (CSF), hippocampus, and frontal cortex of patients with BD, MDD, or schizophrenia have demonstrated differences between these disorders and control subjects.

**N-CAM.** The N-CAM gene is complex with 20 exons and several that can be alternatively spliced leading to at least 27 different mRNA species (Reyes et al 1993). One variation involves the splicing of a 30 bp variable alternative spliced exon (VAS) into N-CAM 140, which is associated to down-regulation of neurite outgrowth (Doherty et al 1992). Another alternative splicing event results in a premature interruption of transcription, which presumably produces a secreted isoform (SEC; Gower et al 1988).

The initial results showed that CSF N-CAM was not changed dramatically in patients with BD (n = 16) compared to control subjects but tended to increase with treatment and remission of depression (Jorgensen et al 1977). CSF N-CAM (100–120 kd) was modestly increased in bipolar I (n = 16) and MDD patients (n = 8), although much larger increases in CSF N-CAM of up to 200% were found in chronic patients with schizophrenia (Poltorak et al 1995, 1996, 1997; van Kammen et al 1998). This CSF N-CAM isoform was later characterized as N-CAM 105–115 kd (Vawter et al 1998a).

MDD patients have a significantly increased total N-CAM in the hippocampus compared to control subjects (32%; Jorgensen and Riederer 1985). A further study of the cytosolic and membrane fractions of hippocampus and BD was conducted (Vawter et al 1998a). The N-CAM isoforms (180, 140, and 120 kd) were normal in BD (n = 6) and schizophrenia (n = 16). The N-CAM isoform (105–115 kd) was elevated only in patients with schizophrenia (+55%) but not in BD (−33%) compared to control subjects (Vawter et al 1998a). The N-CAM 105–115-kd isoform was significantly decreased in BD (−140%) as compared to schizophrenia. Age or postmortem interval differences were not related to differences in N-CAM 105–115 kd between groups. In the hippocampus, N-CAM (105–115 kd) was normal in BD and elevated in schizophrenia, whereas in depressed patients, an elevation was reported of total N-CAM. In CSF, N-CAM 105–120 kd is elevated in MDD, bipolar I, and schizophrenia, whereas bipolar II patients had normal CSF N-CAM 105–120 kd. Taken together, the CSF and hippocampus findings indicate there are alterations in N-CAM, possibly due to the N-CAM 105–115-kd isoform, in MDD and schizophrenia; however, CSF and hippocampus findings in bipolar disorder suggest that some alteration in N-CAM might be found only in bipolar disorder I. The relationship of treatment and response differences between the BD and schizophrenia requires further investigation, a caveat applicable to the findings reported below N-CAM.

**VARIABLE ALTERNATIVELY SPliced EXON (VASE) OF N-CAM.** Variable alternatively spliced exon N-CAM protein expression was examined in BD (n = 6) and schizophrenia (n = 16) by quantitative Western immunoblot (Vawter et al 1998b). Variable alternatively spliced exon immunoreactive proteins were examined in the prefrontal cortex and hippocampus. Cytosolic VASE 140 kd was increased in the hippocampus of patients with BD as compared to control subjects, patients with schizophrenia, and suicide cases. In the prefrontal cortex, cytosolic VASE 140 kd was increased in BD. Membrane-associated VASE 140 kd was not different between groups. In the CSF, VASE 140 kd was normal for BD I (n = 7), BD II (n = 9), MDD (n = 17), and schizophrenia (n = 14), whereas higher molecular weight VASE isoforms (155 and 165 kd) were increased only in the schizophrenia group (Vawter et al 2000). VASE immunostaining colocalized with glial fibrillary acidic protein–positive astrocytes in the hippocampus and VASE immunostaining was also observed in the cytoplasm of CA4 pyramidal neurons (Vawter et al 1998b).

Although speculative, the VASE 140 kd increase in BD in the hippocampus could be related to less brain growth capability, which would be consistent with volume reductions in the hippocampus seen by neuroimaging (Pearlson 1999). The structural changes in the hippocampus have been associated with impairment in neuropsychological functioning (Ali et al 2000). The high level of VASE expression in the adult central as compared with peripheral nervous system could contribute to the poor regenerative capacity of the former (Doherty et al 1992). The absence of the 30 bp VASE has a significantly greater growth-promoting capability than the presence of VASE (Liu et al 1993) in the hippocampus (Forster and Frotscher 1995; Walsh et al 1992). Variable alternatively spliced exon is upregulated also in astrocytes treated with dibutyryl cAMP (Gegelashvili et al 1993). Thus, an increase of VASE 140 in the hippocampus of BD could be related to particular medications that can affect signal pathways, which in turn alter the growth capability of the hippocampus and other brain regions.
SECRETED ISOFORM OF N-CAM (SEC N-CAM). The SEC N-CAM protein was also measured in the hippocampus of BD patients (n = 6) and schizophrenia (n = 16). In BD, but not in schizophrenia, an increased SEC N-CAM 115 kd/108 kd ratio was found as compared to control subjects (Vawter et al 1999). Thus, BD patients show alterations in the expression of the SEC and VASE splice variants of N-CAM in the hippocampus. The biological role of SEC N-CAM in the brain is unknown at this time; however, removal of the cytoplasmic tail of N-CAM in a transgenic mouse results in a lethal embryonic mutation (Rabinowitz et al 1996). Secretoisoform of N-CAM does not possess a cytoplasmic tail and might have a significant role in brain processes, which could be altered in BD.

Other CAMs and Extracellular Matrix Proteins
L1 was not altered in BD (n = 6) in the hippocampus by western immunoblotting (Vawter et al 1998a). Probing of the frontal cortex by immunohistochemistry for Thy-1, another CAM of the immunoglobulin superfamily, and for L1, did not reveal any difference in BD (n = 15; Webster et al 1999).

Tenascin and chondroitin sulfate glycoproteins (CS-56 immunoreactivity) are extracellular matrix molecules (ECMs) thought to be upregulated following seizure activity and regeneration in the hippocampus (Faissner et al 1994; Silver 1994). There were no overall group differences in BD or MDD hippocampal sections (n = 15) for CS-56 or tenascin immunoreactivity (Freed et al 1999). Likewise, no change for BD or MDD were observed in CS-56 or tenascin immunoreactivity in the PFC (BA 46; Johnston, unpublished result). The results suggest little similarity to animal epilepsy models or changes consistent with striking tenascin increases reported in temporal lobe epilepsy patients.

Synaptic Proteins
Synaptic proteins have been shown to be altered in the brains of patients with schizophrenia (for review see Harrison 1999), which suggests a possible decrease in synapse number in the hippocampus. The evidence for alterations in synaptic proteins in BD is limited by fewer reports involving small sample sizes.

Synapsin, a presynaptic marker, is localized to the surface of synaptic vesicles that are both docked and cross-link or tether synaptic vesicles to one another to form a reserve pool of vesicles away from the docking site (Hilfiker et al 1999). Total synapsin was decreased significantly in BD (n = 6) and in patients with schizophrenia (n = 16) as compared to control subjects (Vawter et al, unpublished result). Three synapsin isoforms were reduced in BD (synapsins Ia, Ila, and IIIa) but only reductions in two (synapsin Ila and IIIa) were observed in schizophrenia. By comparison, synaptophysin protein immunoreactivity in the hippocampus did not differ between groups. The reduction in BD of synapsins Ia, Ila, and IIIa protein, but not synaptophysin, suggests that synaptic number might be normal, but specific functions related to neurotransmission may be altered in BD.

The synaptosomal-associated protein 25 kd (SNAP-25), also called D3 protein (Jorgensen 1995), was normal in CSF of BD, although patients with schizophrenia show an increase in CSF SNAP-25 (Thompson et al 1999). In the hippocampus of MDD patients, SNAP-25 was significantly increased compared to control subjects (Jorgensen and Riederer 1985), whereas in the frontal cortex no differences were found in SNAP-25 in MDD. In general, differences in synapsin, synaptophysin, or N-CAM proteins in rats following lithium treatment were not found (Plenge et al 1992). In lithium-fed animals, SNAP-25 was decreased (serum lithium levels of 0.6–0.9 mmol/L) but not in lithium-injected animals (serum lithium of 0.2–0.5 mmol/L; Plenge et al 1992). Studies of SNAP-25 in BD must be carefully evaluated in terms of the medication effect of lithium.

Growth-associated protein (GAP)-43 is thought to be related to synaptic plasticity. A study limited by small groups of bipolar depressed (n = 2) and depressed patients (n = 8) showed a reduction in GAP-43 mRNA and protein in the prefrontal cortex (Hrdina et al 1998) compared with matched control subjects (n = 10). In contrast, GAP-43 was increased in frontal cortex in patients with schizophrenia (Perrone-Bizzozero et al 1996; Sower et al 1995). The interpretation of these preliminary results for bipolar patients is difficult due to small sample size.

An ultrastructural examination of the anterior limbic cortex (BA 24) showed a 43% increase in the density of synapses on dendritic spines (axospinous synapses) of layer II in patients with BD (n = 2) and no change in the axodendritic synapses, compared to control subjects (Aganova and Uranova 1992). Patients with schizophrenia (n = 5) also showed a pronounced increase in axospinous processes, and a significant decrease in axodendritic synapses. Thus, the changes in axospinous synapses are similar in BD and schizophrenia, whereas axodendritic synapses differ in the two disorders.

Although the available data are fragmentary, several studies suggest that a difference in synaptic proteins and N-CAM in the hippocampus and cortex may be involved in the pathophysiology of BD; however, investigation of synaptic function and structure has been undertaken in relatively few anatomic regions and BD patients, thus limiting interpretations of whether a general deficit in
synaptic markers or specific circuits exists in BD. The study of CNS synapses in BD may be a fruitful area for further study.

Summary

The postmortem examination of BD brain has been accelerated in the past decade with the appearance of new findings in areas of pathomorphology, signaling, neuropeptides, neurotransmitters, cell adhesion molecules, and synaptic proteins. The postmortem findings are fragmentary and generally based on small group sizes. As a result, there is insufficient basis for the construction of a cohesive model to integrate these findings. Further, there is concern that selection bias in the postmortem brain collections favors suicide victims, whereas in vivo neuroimaging studies might tend to recruit less severe clinical presentations and use stricter exclusion criteria. The small sample size also leads to a lack of statistical power to detect actual differences between BD patients and control subjects. For example, a typical postmortem BD study with total sample size of 20 subjects will yield minimal power (β = .28), whereas increasing the total sample size to 40–60 subjects will increase the power to moderate to high (β = .54–.74) for detecting a difference between groups (Rose et al 1998). Nevertheless, there are a number of intriguing findings worth pursuing further.

We first summarize the postmortem findings of cell count studies. A decrease in glial number and density were reported in both BD and MDD in the left subgenual PFC. Cortical neuronal counts were not different in BD (BA 9, BA 24); in contrast, in MDD reductions were found in neuronal size and density (BA 9, BA 47). Glial number was normal in the corpus callosum in BD. Moreover, a decrease in nonpyramidal neurons in sector CA2 of the hippocampus was reported in BD and schizophrenia. Finally, an increased number of large pigmented neurons in the LC was reported in BD patients compared to MDD; a trend was noted for an increase in BD patients as compared to control subjects. Clearly, the prefrontal cortical glia have been implicated in BD.

There has also been a report of decreases in gray matter volume, glucose metabolism, and blood flow of the left subgenual PFC in familial BD and familial MDD (Drevets et al 1997). Conversely, the postmortem study that utilized both left and right subgenual PFC samples of familial BD and familial MDD demonstrated a reduction in glial number in each familial mood disorder group (Ongur et al 1998); however, the neuroimaging and postmortem studies are not directly comparable, since the right and left subgenual PFC data in the postmortem study showed a significant decrease in glial counts, whereas the decrease in gray matter volume was specifically found in the left subgenual PFC but not on the right side. (Drevets et al 1997). Nevertheless, in the postmortem study a preliminary data set reported for the left subgenual PFC also showed a trend toward a decrease in gray matter volume in familial BD (n = 4) comparable to the neuroimaging finding (Drevets et al 1997). Thus, the simplest explanation of the gray matter reduction in the left subgenual PFC is that the neuron counts remain normal while a reduction in glia occurs in both familial cases of BD and MDD.

Because only two brain subregions have been systematically investigated by both postmortem and in vivo neuroimaging in BD and MDD, it is speculative to indicate that this reduction in glia and gray matter volume will be selective for only the left subgenual PFC. Further, a presumptive increase in NE-containing terminals in the PFC from the LC might also lead to an increased loss of glia in view of a recent report that norepinephrine potently inhibits proliferation of cortical astrocytes (Kotter and Klein 1999). The loss of glia and impact on neuronal function in BD are further discussed below.

An increase in neuropeptides was found in the PVN of the hypothalamus in BD and MDD brains. There was an extremely small sample with no differences between MDD and BD. Vasopressin-, oxytocin-, and CRH-containing neurons were significantly increased in this group of BD and MDD brains. There was a decrease in neuropeptide Y in BD in the PFC that was not found in schizophrenia or MDD. These results suggest that specific abnormalities in neuropeptides occur in BD (Gold and Goodwin 1978), and further replications in larger samples are clearly required in the hypothalamus.

The concentrations of the neurotransmitters NE, DA, and 5-HT were all normal in BD cortex and thalamus; however, NE turnover (NE/MHPG metabolite ratio) was increased in all areas in BD. The serotonin metabolite, 5-HIAA, and the 5-HT transporter were both reduced in BD. Whether the alterations found in brain serotonin metabolism and transporter binding are indicators of reduced 5-HT could be further investigated by measuring the availability of precursor tryptophan, activity of tryptophan hydroxylase, 5-HT reuptake, and the activity of monoamine oxidase A that preferentially metabolizes 5-HT (Meltzer 1989) in postmortem BD samples. The permissive hypothesis of affective disorders posits an indoleamine reduction and increased catecholamine levels in BD. There are no postmortem neuropathological studies of the NE transporter in BD, which are also needed to test the permissive hypothesis. The increased number of LC neurons reported in BD compared to MDD suggests an alteration of NE in projection regions such as the PFC. If confirmed, this finding would also be consistent with the permissive hypothesis. A direct test of the permissive hypothesis would require examination of multiple brain...
regions from the same cohort that are assayed for several NE and 5-HT precursors, metabolites, and transporters.

In the realm of postreceptor signal transduction, several experiments have implicated both cAMP and PI cascade abnormalities, which can be linked to G protein–coupled receptors. G protein concentrations and activity were increased in occipital, prefrontal, and temporal cortex in BD; however, in BD, there were no differences in mRNA for G proteins in any cortical area examined, suggesting that posttranscriptional regulation of G protein activity, such as conformational changes, might occur that could increase activity and confer regional specificity. Observations in brain and platelets suggest increases in signal transduction via adenylyl-cyclase activity due to an increased coupling to G proteins. In the PI signal cascade, a decrease in inositol was found in BD in the prefrontal cortex but not in the occipital cortex or cerebellum. PI hydrolysis was decreased in occipital cortex, but not frontal or temporal cortex in BD. In frontal cortex PKC activity in membranes was increased, whereas in the cytosol PMA-stimulated PKC activity was reduced in the frontal cortex. Translocation of PKC activity from cytosol to membrane was increased in BD in the frontal cortex. Thus, evidence from several sources suggests that signal transduction pathways are altered in BD; however, the precise form of these alterations, regional differences in brain, and the relation to pathophysiology is not clear. Further, it was found that lithium induced a reduction of inositol in the right frontal cortex before clinical improvement (Moore et al 1999). The effects of lithium and the PI signal cascade in BD are suggested to occur through longer term changes in gene expression. This would be a fruitful area for future investigation through the use of technology such as microarrays for detecting changes in gene expression over the course of lithium administration.

Secreted isoform of N-CAM and VASE N-CAM, which are alternatively spliced exons of the neural cell adhesion molecules, were altered in the hippocampus of BD. These findings are in contrast to schizophrenia, where no differences were found for either of these N-CAM splice variants. Over-expression of VASE leads to a down-regulation of neurite outgrowth. Whether reduced neurite outgrowth and possibly less regenerative capacity in terms of neurogenesis and structural abnormalities is present in the hippocampus of BD requires further investigation.

The synaptic protein markers (synapsin Ia, IIa, IIIa) were found to be reduced in BD, whereas synaptophysin was normal in BD hippocampus. Growth-associated protein 43 was also reduced in the PFC of BD. Axospinous synapses were actually increased in BD but not in schizophrenia, whereas axodendritic synapses appeared to be increased in both disorders. The findings of alterations in N-CAM and synaptic proteins in BD implicate neural plasticity and synaptic remodeling in the pathophysiology of BD occurring in the PFC and hippocampus. A distinct set of findings was seen in schizophrenia, suggesting that the two disorders may involve different forms of abnormality in synapse remodeling or synaptic function; however, medication effects and response differences between the two disorders can not be ruled out in the small sample sizes and requires further investigation.

Two major themes appear in the literature on the neuropathology of BD. First, there appears to be some convergence in the area of reduction in glial numbers and function. The number and density of glia cells appear to be reduced in BD. This suggests a wide range of potential studies on the regulation of glial survival and proliferation in BD. A detailed consideration of the factors involved in regulation of glial number or density is beyond the scope of this review. Briefly, a number of growth factors, cytokines, and chemokines have been shown to influence glial proliferation or migration, such as basic fibroblast growth factor, colony-stimulating factors, and IL-3 (Eclancher et al 1996; Guillemin et al 1996; Sugita et al 1999). Basic fibroblast growth factor caused increased learning in a spatial task accompanied by increased astrocyte density in the hippocampus (Gomez-Pinilla et al 1998). Therefore, there are numerous possible biochemical causes of changes in glial number in BD. It is possible that the experience or the process involved in BD is responsible for changes in glial number. This latter possibility might be examined via comparisons of basic fibroblast growth factor expression in the cortex of BD subjects and control subjects. Another element that might contribute to glial abnormalities in BD is the increased VASE N-CAM, which can reduce neurite outgrowth and was found to be up-regulated in astrocytes upon chronic stimulation with dibutyryl cAMP. Neural cell adhesion molecule release and cortisol have been shown to decrease glial proliferation (Crossin et al 1997; Krushel et al 1998). In the cortex of BD patients, a glial isoform of Na,K-ATPase is decreased, whereas the neuronal isoform of Na,K-ATPase was normal (Rose et al 1998), which is consistent with possible decreases in glial number or activity (Ongur et al 1998). Glial reduction was found in the frontal cortex and not corpus callosum (Nasrallah et al 1983), suggesting that glia in gray matter are selectively affected as compared to oligodendrocytes in white matter. We have recently observed that markers of long-term gliosis (CS-56 and tensacin) are not changed in the hippocampus (Freed et al 1999) or PFC (Johnston, unpublished result) of patients with BD or MDD. Thus, there does not appear to be a long-term increase in gliosis in the brains of patients with BD, although the data must be further analyzed by familial mood disorder groupings.

At this point, however, the available data are highly
Neuropathology of Bipolar Disorder presents the data are far too sparse to allow definite mechanisms to be suggested; however, clarification of the nature of the changes seen in glia populations may permit cell culture models to be employed to examine the relationships between changes in glia numbers, neurotransmission, and changes in signal transduction that have been found in BD.

One noteworthy anatomic connection related to these findings is the projection of the LC to multiple cortical and subcortical regions (Figure 1). There are findings in postmortem studies of alterations in the LC, PFC (notably the subgenual PFC), hippocampus, and hypothalamus. These regions are implicated in various aspects of emotional behavior. A single unitary pathology has not been found in BD. The circuit involving the LC and presumed connections to hypothalamic–striatal–limbic–cortical structures has yielded data supporting alterations in BD. Notably absent are postmortem studies of the basal ganglia structures and BD.

The quality and number of research findings on BD has substantially increased during the past decade; however, the number of BD brains examined appears very limited, in contrast to other neuropsychiatric disorders. Larger numbers of BD brains are needed to develop confidence in the biological insights afforded by the examinations conducted to date, to conduct the necessary replication studies, and to expand upon the reports that have so far been published, so that it becomes possible to develop coherent models of the pathophysiology of BD. Finally, to integrate postmortem studies with in vivo measurements obtained by neuroimaging, a consortium of investigators that can study selected patients by both methods will be an invaluable resource for investigation of the pathophysiology of BD. Until findings from both perspectives can be integrated, it will be difficult or impossible to develop integrated conceptual models that are based on findings from both fields.

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References


Amygdala enlargement in bipolar disorder and hippocampal reduction in schizophrenia: An MRI study demonstrating neuroanatomic specificity. *Arch Gen Psychiatry* 55:663–664.


Raadsheer FC, Hoogendijk WJ, Stam FC, Tilders FJ, Swaab DF (1994): Increased numbers of corticotropin-releasing hor- 
mon expressing neurons in the hypothalamic paraventricular 
nucleus of depressed patients. Neuroendocrinology 60:436– 
444.

motion of Ncam to produce a secreted molecule results in a 
dominant embryonic lethality. Proc Natl Acad Sci U S A 
93:6421–6424.

Rahman S, Li PP, Young LT, Kofman O, Kish SJ, Warsh JJ 
(1997): Reduced [3H]cyclic AMP binding in postmortem 
brain from subjects with bipolar affective disorder. J Neuro-
chem 68:297–304.

Rajkowska G (1997): Morphometric methods for studying the 

Rajkowska G, Miguel-Hidalgo JJ, Wei J, Dilley G, Pittman SD, 
and glial prefrontal cell pathology in major depression. 
Biol Psychiatry 45:1085–1098.

Ressler KJ, Nemeroff CB (1999): Role of norepinephrine in the 
pathophysiology and treatment of mood disorders. Biol Psychiatry 
46:1219–1233.

NCAM splicing events are differentially regulated during rat brain development. Brain Res Mol Brain Res 
17:201–211.

Reznikoff GA, Manaker S, Rhodes CH, Winokur A, Rainbow 
(1986): Localization and quantification of beta-adrenergic 

molecule (NCAM) in development and plasticity of the 

Rose AM, Mellett BJ, Valdes R Jr, Kleinman JE, Herman MM, 
Li R, el-Mallakh RS (1998): Alpha 2 isofrom of the Na,K-
adenosine triphosphatase is reduced in temporal cortex of 

Rush AJ, Giles DE, Schlessier MA, Orsulak PJ, Parker CR Jr, 
Weissenburger JE, et al (1996): The dexamethasone suppres-
57:470–484.

Schuldi Braer AJ (1965): The catecholamine hypothesis of affective 
122:590.

(1998): Reduced tyrosine kinase receptor C mRNA levels in 
the frontal cortex of patients with schizophrenia. Neurosci 

Seckl JR, French KL, O’Donnell D, Meaney MJ, Nair NP, Yates 

Sherman WR, Munsell LY, Gish BG, Honchar MP (1985): 
Effects of systemically administered lithium on phosphoino-
sitide metabolism in rat brain, kidney, and testis. J Neuro-
chem 44:798–807.

Shimon H, Agam G, Belmaker RH, Hyde TM, Kleinman JE 
(1997): Reduced frontal cortex inositol levels in postmortem 
brain of suicide victims and patients with bipolar disorder. 

Silver J (1994): Inhibitory molecules in development and regen-

Singh MM (1970): A unifying hypothesis on the biochemical 

second messengers in the central nervous system. In: Meltzer 
HY, editor. Psychopharmacology: The Third Generation of 

Soares JC, Mann JJ (1997): The anatomy of mood disorders— 
review of structural neuroimaging studies. Biol Psychiatry 
41:86–106.

levels of GAP-43 protein in schizophrenic brain tissues 

Spleiss O, van Calker D, Scharer L, Adamovic K, Berger 
and alpha(2)-subunit mRNA expression in bipolar affective 
disorder. Mol Psychiatry 3:512–520.

Strakowski SM, DelBello MP, Sax KW, Zimmerman ME, Shear 
PK, Hawkins JM, Larson ER (1999): Brain magnetic reso-

Sugita Y, Zhao B, Shankar P, Dunbar CE, Doren S, Young HA, 
Schwartz JP (1999): CNS interleukin-3 (IL-3) expression and 
neurological syndrome in antisense-IL-3 transgenic mice. 

subtype expression defines morphologically distinct classes of 

Swayze VW, Andrews NC, Alliger RJ, Yuh WT, Ehrhardt JC 
(1992): Subcortical and temporal structures in affective dis-
order and schizophrenia: A magnetic resonance imaging study. 
Biol Psychiatry 31:221–240.

Thompson PM, Rosenberger C, Qualls C (1999): CSF SNAP-25 
in schizophrenia and bipolar illness. Neuropsychopharmacol-

van Kammen DP, Poltorak M, Kelley ME, Yao JK, Gurklis JA, 
spinal fluid neuronal cell adhesion molecule in schizophrenia. 

Vawter MP, Cannon-Spoo HE, Hemperly JJ, Hyde TM, 
VanderPutten DM, Kleinman JE, Freed WJ (1998a): Abnor-
mal expression of cell recognition molecules in schizophrenia. 
Exp Neurol 149:424–432.

Vawter MP, Hemperly JJ, Hyde TM, Bachus SE, VanderPutten 
isotypes are increased in the hippocampus in bipolar disorder 
but not schizophrenia. Exp Neurol 154:1–11.

Vawter MP, Howard AL, Hyde TM, Kleinman JE, Freed WJ 
(1999): Alterations of hippocampal secreted N-CAM in 
bipolar disorder and synaptophysin in schizophrenia. Mol Psychiatry 4:467–475.

Vawter MP, Frye MA, Hemperly JJ, VanderPutten DM, Usen N, 
VES isoforms in schizophrenia. J Psychiatr Res 34:25–34.

Verebey K, Volavka J, Clouet D (1978): Endorphins in psychiat-
ry: An overview and a hypothesis. Arch Gen Psychiatry 
35:877–888.


