Contributions from brain imaging to the elucidation of pathophysiology of bipolar disorder

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Abstract
Over the past two decades, brain-imaging studies have examined the mechanisms possibly involved in the pathophysiology of bipolar and unipolar mood disorders. The available findings suggest subtle anatomical changes in sub-regions of the prefrontal cortex, medial temporal lobe and cerebellum, and functional abnormalities in brain circuits inter-connecting these same brain regions and the striatum in patients suffering from bipolar disorder. $^1$H magnetic resonance spectroscopy (MRS) studies reported decreased N-acetyl aspartate (NAA) levels in the dorsolateral prefrontal cortex, and $^{31}$P-MRS studies found abnormalities in membrane phospholipids in frontal and temporal regions in bipolar individuals. Few studies have utilized in-vivo receptor imaging to study bipolar patients. Even though preliminary findings from cross-sectional studies indicate anatomical, neurochemical, and functional brain abnormalities in bipolar patients in key regions involved in mood regulation, the relationship of such abnormalities with illness phase and their clinical relevance needs further investigation. The potential for utilization of brain-imaging tools to elucidate the pathophysiology of bipolar disorder is still largely unrealized, and it is anticipated that important new developments in this area will come about over the next years and beyond.

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Introduction
Advances in brain-imaging modalities have brought unprecedented possibilities for in-vivo studies of the human brain. Nowadays, it is feasible to examine the in-vivo human brain in the context of anatomical, neurochemical, and functional investigations, with high resolution. Over the past two decades, several studies have examined possible brain mechanisms involved in the pathophysiology of mood disorders (Bearden et al., 2001; Drevets, 2000, 2001; Pearlson, 1999; Soares, 2002; Soares and Innis, 2000; Strakowski et al., 2000, 2002). Magnetic resonance imaging (MRI) studies found subtle anatomical abnormalities in regions involved in brain circuits that participate in mood regulation, such as sub-regions of the prefrontal cortex, medial temporal lobe, striatum, and cerebellum (Drevets et al., 1992, 1997; Mayberg, 1997; Soares and Mann, 1997a, b). The particular neuroatomic circuits that are involved comprise two interrelated brain circuits, a limbic thalamic cortical circuit, and a limbic striatal pallidal thalamic one. Subsequently, functional and neurochemical abnormalities in these brain regions were examined with functional imaging tools, such as single photon emission computerized tomography (SPECT), positron emission tomography (PET), functional magnetic resonance imaging (fMRI), and magnetic resonance spectroscopy (MRS).

In the present paper, we have reviewed the published brain-imaging findings in bipolar disorder patients. All in-vivo human studies that utilized brain-imaging tools to investigate bipolar patients were identified, but papers were selected for inclusion in this review according to their perceived importance.
We summarized available findings and proposed specific strategies for future studies in this field.

Anatomical findings

In-vivo MRI studies reported abnormalities in subregions of the prefrontal cortex in bipolar patients. Sax et al. (1999) found decreased prefrontal cortex volumes in manic patients. Decreased grey-matter volumes in left subgenual prefrontal cortex in familial unipolar mood-disorder and bipolar subjects were reported by Drevets et al. (1997) and Hirayasu et al. (1999). Nonetheless, two recent studies failed to identify abnormalities in grey-matter volume in subgenual prefrontal cortex in bipolar (Brambilla et al., 2002) and unipolar (Brambilla et al., 2002; Bremner et al., 2002) patients. Figure 1 illustrates the tracing of the subgenual prefrontal cortex on an MRI. Abnormalities in the subgenual prefrontal cortex were further confirmed in neuropathological studies, with findings of decreased glia without a corresponding loss in neuronal density or size (Ongur et al., 1998). Rakjowska et al. (2001) found reduced neuronal size and density, and reduced glial density in the dorsolateral prefrontal cortex (DLPFC) in post-mortem brains of bipolar patients. These findings are in line with functional imaging literature demonstrating decreased blood flow and metabolism in the DLPFC in mood-disorder subjects (Soares and Mann, 1997b). Subsequently, Sassi et al. (2002) reported a significant reduction in grey-matter volumes in left anterior cingulate in untreated bipolar disorder patients compared to healthy controls. Figure 2 illustrates the tracing of the anterior cingulate on an MRI. Interestingly, in this particular study, the grey-matter values in the left anterior cingulate of lithium-treated bipolar patients were not reduced compared to healthy controls, suggesting that lithium could possibly have the effect of preventing or reversing grey-matter changes in this particular brain region. The preliminary findings for the anterior cingulate will be further examined in controlled longitudinal studies involving larger patient samples, as well as studies in first-break and early-onset cases of the illness.

In regard to temporal lobe abnormalities, the most consistent findings relate to enlargement of the amygdala. Two published studies found increased amygdala volumes in bipolar patients compared to healthy controls (Altshuler et al., 1998; Strakowski et al., 1999), which is in agreement with our recently published findings of enlarged left amygdala in bipolar patients (Brambilla et al., 2001b). Figure 3 illustrates the tracing of the amygdala on an MRI. Most studies that measured the hippocampus in bipolar patients failed to identify abnormalities (Brambilla et al., 2001b; Hauser et al., 1989; Strakowski et al., 1999), with the exception of one that found decreased right hippocampal volumes (Swayze et al., 1992). This is in contrast to available findings in unipolar disorder, where most studies found decreased hippocampal volumes (Sheline, 2000; Sheline et al., 1996, 1999; Soares and Innis, 2000). A reduction in hippocampal size may be mediated by dysfunction in the HPA axis and be possibly related to hypercortisolaemia.

Most studies in bipolar patients did not find abnormalities in caudate or putamen (Brambilla et al., 2001c; Soares and Mann, 1997a), while studies in unipolar disorder have shown smaller caudate and putamen

Figure 1. An illustration of the tracing of the subgenual prefrontal cortex on an MRI, bilaterally.
volumes (Husain et al., 1991; Krishnan et al., 1991; Parashos et al., 1998; Shah et al., 1998). These findings suggest that anatomical abnormalities in the striatum may be more characteristic of unipolar disorder.

The thalamus has also been a brain region of interest, as it is a key brain structure that inter-connects various cortical and sub-cortical regions. There are reports of reduction in thalamic size in schizophrenic and first-break psychotic patients (Byne et al., 2001; Gilbert et al., 2001; Konick and Friedman, 2001). However, the available findings involving bipolar disorder patients largely suggest that, anatomically, this structure is not affected (Caetano et al., 2001; Soares and Mann, 1997a).

Anatomical abnormalities in the cerebellum (vermis atrophy and decreased cerebellar size) have been reported in several computerized tomography (CT) studies in bipolar and unipolar subjects (Soares and Mann, 1997a). In a recent report by DelBello et al. (1999), atrophy in the V3 vermal area was found in multiple-episode, but not in first-episode bipolar patients. Brambilla et al. (2001a) examined a group of bipolar and unipolar mood-disorder patients and did not find evidence of abnormalities in vermis or cerebellar size. Anatomical abnormalities in the cerebellum, if present, could constitute a neurodegenerative process, and possibly be of relevance for mood regulation because the cerebellum has anatomical projections to the brainstem and limbic regions.

Functional findings
The functional imaging studies in mood disorders published to this date have largely included SPECT and PET studies conducted during resting state. PET investigations that utilized $^{15}$O water and $^{18}$F FDG to examine cerebral blood flow and glucose metabolism, respectively, have found hypofrontality in unipolar and bipolar individuals. Functional abnormalities in depressed patients in the anterior cingulate have been reported (Drevets et al., 1997; Mayberg, et al., 1994). Drevets et al. (1997) found decreased blood flow and glucose metabolism in the subgenual prefrontal cortex of familial unipolar and bipolar patients. Dolan et al. (1994) reported a functional deficit in medial prefrontal cortex in mood-disorder patients, which was related to neuropsychological dysfunction. George et al. (1997) found blunted left cingulate activation.
at the Stroop test, indicating dysfunction in attention mechanisms. In a preliminary fMRI study in bipolar patients, Blumberg et al. (1999) reported evidence of dysfunction in rostral and orbital prefrontal cortex regions. In summary, available functional findings in bipolar and unipolar mood-disorder patients suggest involvement of abnormalities in the prefrontal cortex in the pathophysiology of these illnesses. Increased blood flow has been reported in the left amygdala in familial unipolar disorder subjects in a PET [18O] water study (Drevets et al., 1992). Sheline et al. (2001) found evidence of abnormally responsive left amygdala when unipolar depressed patients were submitted to emotional stimuli with a face recognition task. This abnormality was resolved as a result of antidepressant treatment. Decreased blood flow and glucose metabolism in basal ganglia, particularly caudate nuclei, have been reported in several studies, which involved largely unipolar subjects (Soares and Mann, 1997b).

In summary, available findings from the functional imaging studies that involved bipolar patients have been somewhat unclear. Because the majority of the available studies has not been longitudinal, and has not examined the same patients across different mood states, the relevance of the identified abnormalities for the pathophysiology of the illness remains uncertain. For instance, it is not well-established whether identified abnormalities in frontal and limbic regions are trait or state changes. Longitudinal studies involving untreated individuals in various mood states will be needed to clarify the relationship between such abnormalities and the symptoms of the illness. Nonetheless, those will be very challenging studies to complete, due to difficulties in following these patients over time and successfully completing brain scans repeatedly over different mood states. In particular, the manic phase of the illness has been largely understudied, as a result of the feasibility problems in being able to successfully complete brain scans in patients that are often agitated and non-cooperative during the manic state.

**In-vivo neurochemical findings**

**MRS studies**

In-vivo 1H-MRS of the brain has been utilized, over the past few years, as an important new tool for investigations in mood disorder subjects (Soares et al., 1996). Figure 4 illustrates the results of a 1H-MRS human brain study. N-acetyl aspartate (NAA) is a chemical that can be measured with 1H-MRS, and is a non-specific marker of neuronal viability and/or function. Its levels may be reduced in particular brain regions in mood-disorder subjects, and such reductions would indicate abnormal neuronal processes locally. Winnberg et al. (2000) reported decreased levels of NAA in the DLPFC in bipolar patients. Further evidence for reduced levels of NAA in the DLPFC in bipolar adolescents was provided by two different groups (Chang et al., 2001; Sassi et al., 2001). Moore et al. (2000b), in a preliminary study that involved a relatively small group of bipolar patients and healthy controls, found that lithium treatment resulted in increased NAA levels in various cortical regions. Considering the reported effects of lithium in increasing production of neurotrophic factors (Manji et al., 2000), these findings could represent in-vivo evidence in support of its neuroprotective effects. Nonetheless, they are still preliminary and will require further confirmation in longitudinal studies with larger patient samples.

Choline is a component of cell membranes, and has important roles in membrane function. With in-vivo 1H-MRS, a choline peak can be detected. This peak contains various choline-containing molecules, and free choline is a small portion of it. Increased choline peak in basal ganglia has been reported in bipolar disorder subjects (Soares et al., 1996). Moore et al. (2000a) reported increased Cho/PCr-Cr ratios in the right anterior cingulate in bipolar patients. Increased Cho/PCr-Cr ratios were also reported in the orbitofrontal cortex in depressed adolescents (Steingard et al., 2000). Renshaw et al. (1997) found significantly reduced Cho/PCr-Cr ratios in basal ganglia in depressed unipolar subjects; this reduction was more pronounced in subjects who responded to treatment with fluoxetine. The increase in choline-containing molecules in basal ganglia reported in some studies with bipolar patients does not seem due to lithium treatment, as it is also present in drug-free patients. This is an important area where further development is clearly needed; additional studies in unmedicated patients, and improved methodology to quantitate free choline in in-vivo MRS studies will be important developments to further elucidate the significance of suggested abnormalities.

Abnormalities in the intracellular phosphoinositol (PI) pathway in brain neurons have been hypothesized to be implicated in pathophysiology of bipolar disorder (Soares and Mallinger, 1997). Lithium has important effects in this pathway, and such effects may be critical mechanisms involved on its therapeutic actions (Soares et al., 2000). Myo-inositol is a substrate for recycling of inositol phospholipids in the PI pathway. Moore et al. (1999) reported, in a 1H-MRS study involving bipolar patients, that myo-inositol levels in the right frontal lobe were significantly
decreased after 4 wk of lithium treatment. Davanzo et al. (2001) provided further corroboration for these findings, and reported bilateral reductions in myo-inositol levels in anterior cingulate in a sample of bipolar adolescents. Future clinical research studies that will attempt to clarify the possible relevance of abnormalities in brain myo-inositol levels and functioning of the PI pathway in the pathophysiology of bipolar disorder and mechanisms involved in treatment response to lithium will be extremely important.

A recent in-vivo 1H-MRS study in unipolar depressed subjects reported increased glutamine and glutamate (Glx peak) in the DLPFC (Brambilla et al., 2001d). In a group of depressed bipolar adolescents, an increase in the Glx peak in the frontal cortex, bilaterally, as well as basal ganglia has recently been reported (Castillo et al., 2000). A hyperglutamatergic state in some of the key brain regions involved in mood regulation could possibly be a mechanism involved in the pathophysiology of mood disorders, and such hypothesis should be specifically examined in future studies. Increased glutamate levels could be neurotoxic, and such abnormality could possibly result in anatomical abnormalities in the prefrontal cortex reported in brain-imaging and post-mortem studies (Rajkowska et al., 2001; Soares and Mann, 1997b). However, one potential limitation of available findings is that the 1H-MRS method utilized in these preliminary in-vivo studies did not allow optimal resolution of glutamate and glutamine. Future studies utilizing improved MRS methods that would allow this distinction will be quite important. Sanacora et al. (1999) conducted an in-vivo 1H-MRS study of the occipital cortex and reported decreased GABA levels in untreated depressed unipolar subjects. These same authors also reported that treatment with electroconvulsive therapy or selective serotonin reuptake inhibitors (SSRIs) resulted in significant increase in GABA levels in the occipital cortex (Sanacora et al., 2002). Such findings indicate the involvement of GABAergic brain mechanisms in the pathophysiology of mood disorders. Nonetheless, available studies are preliminary and will require further replication and examination of other brain regions.

31P-MRS provides an important new tool for in-vivo studies of neuronal membrane processes and metabolism. Kato et al. (1993, 1994) reported increased levels of phosphomonoesters (PME) in the manic and depressed phases, and decreased PME in the euthymic phase. Deicken et al. (1995a, b) found decreased PME in frontal and temporal lobes in euthymic bipolar individuals. PMEs are precursors of membrane phospholipid metabolism, while PDEs are breakdown products. The reported abnormalities in PMEs in bipolar patients could be consistent with increased membrane anabolism in the frontal and temporal lobes in the manic and depressed phases, and decreased in the euthymic one. Nonetheless, it is not clear in these studies whether increased levels of PME could have been attributed to medication effects, as patients were mostly on lithium, or off lithium for

Figure 4. In-vivo 1H-MRS spectrum collected from human left dorsolateral prefrontal cortex. PCr+Cr = creatine + phosphocreatine, Gln = glutamine, Glu = glutamate, INO = inositol, NAA = N-acetyl-aspartate, and TMA = trimethylamines (which includes mainly phosphorylcholine and glycerophosphocholine).

Figure 5. The figure illustrates a PET [11C]flumazenil scan of the human brain. The image shows the distribution of GABA A/benzodiazepine receptors.
short periods. Kato et al. (1998) also reported decreased pH in the frontal lobe of lithium-treated and drug-free euthymic bipolar subjects. In areas of increased white-matter hyperintensities, decreased pH and increased PDE were reported (Kato et al., 1998). Decreased pH and increased white-matter hyperintensities are non-specific findings that are possibly related to various types of brain insults. Future studies should attempt to replicate these preliminary findings in larger groups of unmedicated bipolar patients.

**Receptor imaging studies**

Preliminary in-vivo investigations of the serotonergic brain system of mood-disorder patients have recently been reported. Malison et al. (1998) reported decreased density of serotonin transporters (5-HTT) in the midbrain in drug-free depressed unipolar subjects. However, with the radiotracer utilized in this study ($^{11}$C)IBZM or $^{123}$I]epidepride, examination of 5-HTT levels in cortical regions is not feasible. To our knowledge, no studies have examined 5-HTT levels in bipolar individuals. With the development of new PET radiotracers, such as $^{11}$C]MeCN5652, the examination of 5-HTT levels in cortical regions has become methodologically feasible. Other studies have focused on the 5-HT-2A receptor system. Biver et al. (1997) reported decreased $^{18}$F]altanserin binding in the right postero-lateral, orbitofrontal, and anterior insular cortex in untreated unipolar depressed patients, which is consistent with decreased 5-HT-2A receptor levels. Massou et al. (1997) reported increased 5-HT-2A binding in the frontal cortex of SSRI-treated compared to untreated depressed patients in a PET $^{18}$F]setoperone study (Massou et al., 1997), while Attar-Levy et al. (1999), in another PET $^{18}$F]setoperone study, did not find abnormalities in 5-HT-2A cortical levels, with the exception of reduced binding in frontal cortex. Meyer et al. (1999) reported normal density of 5-HT-2A receptors in unipolar patients in another PET $^{18}$F]setoperone study, while Yatham et al. (2000) reported decreased 5-HT-2A binding in several cortical regions in unmedicated depressed subjects, in a PET study utilizing this same tracer. In summary, the available results for changes in 5-HT-2A receptors are conflicting, and are largely in disagreement with post-mortem findings, where increased 5-HT-2A cortical levels in suicide and in mood disorders had been reported. The 5-HT-1A receptor system has been studied in in-vivo human studies with the PET tracer $^{11}$C]carbonylWAY100635. Drevets et al. (1999) found reduced levels of 5-HT-1A receptors in mesiotemporal cortex, hippocampus, pregenual anterior cingulate, and lateral orbitocortex in depressed familial unipolar and bipolar patients, while Sargent et al. (2000) found reduced binding potential in frontal, temporal, and limbic cortex in SSRI-treated and unmedicated depressed unipolar individuals. With availability of appropriate in-vivo methodology for studies of 5-HT-2A and 5-HT-1A receptors, and 5-HTT, future investigations should allow comprehensive examination of the involvement of serotonergic abnormalities in the pathophysiology of mood disorders and the mechanisms of action of antidepressants.

A few preliminary studies have started to investigate the hypothesis of involvement of the dopaminergic system in the pathophysiology of mood disorders. Pearlson et al. (1995), in a PET study with the radiotracer $N$-[1$^3$C]methylspiperone ($^{13}$C]NMSP), found increased binding potential in the striatum in neuroleptic-naive and neuroleptic-free psychotic bipolar patients compared to non-psychotic ones and to normal controls. These findings are consistent with increased striatal D2 receptor levels, and are similar to findings in schizophrenic patients. Anand et al. (2000), on the other hand, did not find abnormalities in D2 striatal baseline levels, nor on amphetamine-induced dopamine release, in euthymic lithium-treated bipolar patients. However, the sample involved in this study consisted of patients who were euthymic, non-psychotic, and currently medicated. The available findings in bipolar patients are still quite limited and should be seen as preliminary, but suggest that abnormalities in dopaminergic brain pathways may be characteristic of psychotic patients. In summary, very few in-vivo brain-imaging studies examined D2 receptors in bipolar patients; nonetheless, there is a suggestion from one study that neuroleptic-free and neuroleptic-naive psychotic bipolar patients have increased D2 receptor levels in the striatum. Future investigations should also examine the levels of D2 receptors in extra-striatal regions, with improved radiotracers such as the SPECT tracer $^{11}$C]epidepride, or the PET tracer $^{11}$F]fallypride. The SPECT tracer $^{11}$C]flballypride also allows quantitation of dopamine transporters in the striatum; one study by Malison et al. (1998) yielded negative findings in unipolar patients. Nonetheless, no available studies to date have examined dopamine transporters in medication-free bipolar individuals. Recently, a pharmacological paradigm with $\alpha$-methyl-paratyrosine (AMPT) and $^{123}$I]-IBZM or $^{123}$I]raclopride has been utilized to study baseline dopamine levels. Such investigations are of great interest due to findings of increased dopamine release in schizophrenics, which may be a marker of psychotic states (Soares and Innis, 1999). Nonetheless,
preliminary studies that applied these methods for investigations in depressed unipolar subjects have produced negative results (Parsey et al., 2001). In summary, only two published in-vivo brain-imaging studies have examined the dopaminergic system in bipolar patients, and one of these studies suggested dysfunction, with elevated levels of D2 receptor in the striatum in psychotic bipolar patients. Future studies involving untreated patients in different mood states will be required to clarify any involvement of dopaminergic brain abnormalities in the pathophysiology of mood disorders.

The GABAergic system is also of potential relevance for the pathophysiology of mood disorders. With the availability of tracers such as PET \([^{11}C]\)flumazenil, it is feasible to investigate certain aspects of the GABAergic system (GABA A/benzodiazepine receptors), and to examine its potential involvement in the pathophysiology of mood disorders, as well as the mechanisms of action of anticonvulsant medications that are effective for the treatment of bipolar disorder. Brain-imaging studies examining the involvement of the GABAergic system in bipolar and unipolar mood disorders are currently underway (Figure 5).

Pharmacological fMRI

fMRI, in conjunction with specific pharmacological paradigms, has been utilized for in-vivo neurochemical studies of the human brain (Salmeron and Stein, 2002; Stein, 2001). However, such studies are still largely in a preliminary state, as specific pharmacological paradigms for utilization with fMRI are beginning to be developed. There is clearly a very interesting untapped potential for utilization of pharmacological fMRI for neurochemical studies in this area, and we should start seeing, increasingly, the utilization of such tools for investigations in mood disorders. Such studies are able to go beyond simple receptor quantification, and could provide a functional measurement of specific neurotransmitter pathways in the brain. Therefore, the potential usefulness of these new applications of fMRI methodology is very promising.

Conclusions

Bipolar patients have detectable in-vivo anatomical, neurochemical, and functional abnormalities in neuroanatomic brain circuits involved in mood regulation. The prefrontal cortex, medial temporal lobe, striatum, cerebellum, and circuits interconnecting these brain regions appear to be affected. MRI studies with improved resolution have allowed careful investigations of the neuroanatomy of mood disorders. New fMRI studies have started to characterize the functional brain impairment present in these patients. The potential of pharmacological fMRI is beginning to be explored, but there are as of yet no specific reports involving mood-disorder patients. The availability of improved methods for in-vivo neurochemical studies, as well as methods for imaging neurotransmitters and neurotransmitter levels in the in-vivo human brain have provided the tools that will allow comprehensive investigations of the role of specific neurotransmitter systems in the pathophysiology of mood disorders. However, this potential is still largely unrealized, as existing findings in this field are in a very preliminary state.

The available findings in this field suffer largely from important methodological shortcomings. First, findings originated largely from cross-sectional studies. Secondly, most available studies included relatively small patient samples, or samples where patients were, in the majority, on psychotropic medications that could have confounded the results. Last, very heterogeneous imaging methods have been utilized. For these reasons, results are often conflicting, and there is still an important need for independent replication of most findings. Longitudinal studies that will examine the influence of mood and medication effects on in-vivo brain-imaging abnormalities are much needed. Furthermore, studies that will concentrate on first-break and early-onset cases of the illness, as well as high-risk populations will also be very important.

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