Dissertação de mestrado

AVALIAÇÃO IN VIVO DO METABOLISMO DO CÓRTEX PRÉ-FRONTAL DORSOLATERAL EM PACIENTES BIPOLARES EM EPISÓDIO MANÍACO: UM ESTUDO COM ESPECTROSCOPIA POR RESSONÂNCIA MAGNÉTICA

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“Nós devemos lembrar que todas nossas idéias provisórias da psicologia serão, presumivelmente, um dia baseadas em uma subestrutura orgânica.”

Sigmund Freud (1914)
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Resumo

**Introdução:** Diversas anormalidades neuroquímicas têm sido relatadas no Transtorno Bipolar (TB), mas os verdadeiros mecanismos envolvidos na fisiopatologia do TB permanecem a ser elucidados. A técnica de espectroscopia por ressonância magnética ($^1$H-MRS) permite a mensuração de certos neurometabólitos no cérebro humano *in vivo*. Nós utilizamos a $^1$H-MRS para investigar o N-acetil-L-aspartato (NAA), compostos de colina (Cho), creatina/fosfocreatina (Cr) e o *myo*inositol (Ino) no córtex pré-frontal dorsolateral (CPFDL) em indivíduos bipolares durante episódio maníaco/misto.

**Métodos:** Dez pacientes bipolares (9 maníacos, 1 misto), diagnosticados através de uma entrevista clínica semi-estruturada (SCID), e 10 voluntários normais pareados por sexo e idade foram estudados. Os neurometabólitos foram mensurados através de voxels de $8\text{cm}^3$ localizados no CPFDL direito e esquerdo para aquisição da $^1$H-MRS de 1.5T. Imagens de ressonância magnética anatômica ponderadas em T1 e T2 foram obtidas para excluir quaisquer anormalidades neuroanatômicas.

**Resultados:** Não foram encontradas diferenças significativas para NAA, Cho, Cr, Ino, NAA/Cr, Cho/Cr, ou Ino/Cr entre pacientes e controles. Pacientes maníacos/mistos apresentaram níveis significativamente aumentados de *myo*inositol no CPFDL esquerdo em relação ao CPFDL direito ($p = 0.044$).

**Conclusões:** Elevação do *myo*inositol no CPFDL esquerdo em pacientes bipolares durante mania aguda pode representar uma disfunção na via de sinalização do fosfatidilinositol. Estudos longitudinais com maior amostra avaliando o pré e pós-tratamento são necessários para melhor esclarecer este tema.
Abstract

**Background:** Several neurochemical abnormalities have been reported in bipolar disorder (BD), but the exact mechanisms that underlie the pathophysiology of BD remains to be elucidated. The proton magnetic resonance spectroscopy (1H-MRS) technique allows measuring of certain neurometabolites, in human brain in vivo. We used 1H-MRS to investigate dorsolateral prefrontal cortex (DLPFC) N-acetyl-L-aspartate (NAA), choline-containing compounds (Cho), creatine/phosphocreatine (Cr), and myo-inositol (Ino) in bipolar subjects during manic/mixed phase.

**Method:** Ten bipolar patients (9 manic, 1 mixed), diagnosed by a semi-structured clinical interview (SCID), and 10 age- and gender-matched healthy volunteers were studied. Neurometabolites were measured with 8cm³ voxels placed in left and right DLPFC for acquisition of 1H-MRS at 1.5T. T1- and T2-weighted anatomical magnetic resonance imaging was performed to exclude any neuroanatomical abnormality.

**Results:** No significant differences were found for NAA, Cho, Cr, Ino, NAA/Cr, Cho/Cr, or Ino/Cr between patients and controls. Manic/mixed patients had significantly higher left-to-right myo-inositol levels in DLPFC (p = 0.044).

**Conclusions:** Increased myo-inositol in the left DLPFC in bipolar patients during acute mania may represent a dysfunction in the phosphoinositide-signaling pathway. Longitudinal studies with larger samples assessing pre and posttreatment times are required for further clarify this matter.
Introdução e Objetivos da Dissertação

O Transtorno Bipolar (TB) é uma doença altamente incapacitante, que acomete cerca de 1% da população em nosso meio. O TB tipo I caracteriza-se pela ocorrência de, pelo menos, um episódio maníaco ou misto durante a vida, freqüentemente acompanhado de episódios depressivos recorrentes. O diagnóstico de episódio maníaco se dá pela presença de um período distinto de humor anormal, persistentemente elevado, expansivo ou irritável, com duração mínima de uma semana, ou com qualquer duração se a hospitalização for necessária. Além disso, durante o período de perturbação do humor 3 ou mais dos seguintes sintomas devem estar presentes em grau significativo (4 ou mais se humor for apenas irritável): auto-estima elevada ou grandiosidade, diminuição da necessidade do sono, estar mais falante que o habitual ou apresentar pressão para falar, fuga de idéias ou sensação subjetiva de aceleração do pensamento, distratibilidade, aumento da atividade dirigida a objetivos (socialmente, no trabalho, na escola, ou sexualmente) ou agitação psicomotora e envolvimento excessivo em atividades prazerosas com um alto potencial para conseqüências negativas (p. ex. surtos incontrolados de compras ou indiscricções sexuais). A alteração do humor deve ser suficientemente severa para causar marcado prejuízo no funcionamento ocupacional, social ou no relacionamento com os outros, ou necessitar hospitalização, ou apresentar sintomas psicóticos. O diagnóstico de episódio misto, por sua vez, se dá pela presença concomitante de sintomas que satisfaçam os critérios para episódio maníaco e episódio depressivo maior (exceto pela duração), por um período mínimo de uma semana. O episódio depressivo maior caracteriza-se pela presença de cinco ou mais dos seguintes sintomas, durante um período mínimo de duas semanas: humor deprimido durante a maior parte do dia, praticamente todos os dias, diminuição do
interesse ou prazer nas atividades, perda ou ganho significativo de peso (> 5% do peso corporal em um mês) ou diminuição ou aumento do apetite, insônia ou hipersonia quase todos os dias, agitação ou retardo psicomotor, fadiga ou diminuição de energia, sentimento de inutilidade ou culpa ou culpa excessiva ou inadequada, diminuição da capacidade de pensar ou se concentrar ou de tomar decisões e pensamentos recorrentes de morte, ideação ou tentativa de suicídio. Humor deprimido ou perda do interesse ou prazer nas atividades devem estar presentes para o diagnóstico.

Diversos modelos têm sido propostos para explicar as possíveis bases biológicas relacionadas à modulação do humor, sendo que as principais teorias que buscam elucidar a fisiopatologia dos transtornos de humor originaram-se a partir dos estudos dos mecanismos de ação dos fármacos utilizados no tratamento destes transtornos. De fato, os estudos farmacológicos têm demonstrado ação dos antidepressivos e estabilizadores de humor em diversos mecanismos intracelulares que envolvem a regulação da expressão gênica e da plasticidade celular. Possivelmente, uma das dificuldades dos modelos fisiopatológicos do TB é de que as regiões envolvidas na regulação do humor ainda não estão completamente determinadas, sendo que estudos de neuroimagem têm proposto um modelo neuroanatômico de regulação do humor, que compreende interconexões entre o córtex pré-frontal, complexo amígdala-hipocampo, tálamo e os gânglios basais, sendo que, de acordo com este modelo, os transtornos de humor poderiam resultar de uma disfunção em diferentes partes deste circuito. Alterações neuroquímicas em distintas regiões do córtex pré-frontal são consistentes com as características clínicas observáveis em indivíduos bipolares, uma vez que o córtex pré-frontal é considerado como uma das regiões envolvidas na regulação do humor, da motivação, da volição e da memória de trabalho (working memory).
O objetivo deste estudo é determinar os níveis de N-acetil-L-aspartato (NAA), compostos de colina (Cho), creatina/fosfocreatina (Cr) e myoínsitol (Ino) no córtex pré-frontal dorsolateral de 10 pacientes em episódio maníaco, verificando se esses níveis são significativamente diferentes de um grupo-controle de 10 indivíduos normais, pareados por sexo e idade. Nossa hipótese é de que existem alterações neuroquímicas no córtex pré-frontal dorsolateral de indivíduos bipolares em episódio maníaco/misto, que podem ser demonstradas pela técnica de espectroscopia por ressonância magnética. De acordo com nossa hipótese, esperamos encontrar uma diminuição significativa do NAA, o que traduziria um estado de disfunção neuronal local, e um aumento significativo do myoínsitol, o que refletiria uma possível alteração na cascata de sinalização intracelular do segundo-mensageiro fosfatidilinositol.
A PROTON MAGNETIC RESONANCE SPECTROSCOPY INVESTIGATION OF
THE DORSOLATERAL PREFRONTAL CORTEX IN ACUTE MANIA

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ABSTRACT

**Background:** Several neurochemical abnormalities have been reported in bipolar disorder (BD), but the exact mechanisms that underlie its pathophysiology remain to be elucidated. Proton magnetic resonance spectroscopy ($^1$HMRS) technique allows *in vivo* measurements of certain neurometabolites, in the human brain. We used $^1$HMRS to investigate the dorsolateral prefrontal cortex (DLPFC) in bipolar subjects during a manic or mixed phase. N-acetyl-L-aspartate (NAA), choline-containing molecules (Cho), creatine plus phosphocreatine (Cr), and *myo*inositol (Ino) were measured.

**Method:** Ten bipolar patients (9 manic, 1 mixed), diagnosed by a semi-structured clinical interview (SCID), and 10 age- and gender-matched healthy volunteers were studied. Absolute neurometabolites levels were measured from two 8cm$^3$ voxels placed in left and right DLPFC using a short TE $^1$HMRS method at 1.5T. T1- and T2-weighted anatomical magnetic resonance imaging was performed to exclude any neuroanatomical abnormality.

**Results:** No significant differences were found for NAA, Cho, Cr, Ino, NAA/Cr, Cho/Cr, or Ino/Cr between patients and controls. Manic/mixed patients had significantly higher left-to-right *myo*inositol ratios in DLPFC ($p = 0.044$).

**Conclusions:** Increased left-to-right *myo*inositol ratios in the DLPFC in bipolar patients during acute mania may represent a dysfunction in the phosphoinositide-signaling pathway. Longitudinal studies with larger samples of unmedicated patients assessing pre and post-treatment times will be required for further
clarification of the time course of these abnormalities and relationship with treatment effects.

**KEY WORDS** – Bipolar disorder; mania; dorsolateral prefrontal cortex; proton magnetic resonance spectroscopy; mood disorders; neuroimaging
INTRODUCTION

Bipolar disorder (BD) is associated with an increased risk of suicide and disability (Goodwin and Jamison, 1990) and affects up to 1.6% of the population worldwide (Blazer, 2000). Although considered a brain-based disorder, the pathophysiology of BD is poorly understood. Structural and functional neuroimaging studies have suggested that mood disorders could result from dysfunction in cortical-thalamic-limbic circuits, including prefrontal and temporal cortex, amygdala, hippocampus, thalamus, cerebellum, and their interconnections (Soares and Mann, 1997; Strakowski et al., 2000). Postmortem studies have demonstrated a 41% decrease of glial cells in the subgenual prefrontal cortex (PFC) of bipolar patients with a positive family history for mood disorder (Öngur et al., 1998), and a significant reduction in neuronal density in the dorsolateral prefrontal cortex (DLPFC) (Rajkowska et al., 2001), hippocampal CA2 sector (Benes et al., 1998), and anterior cingulate cortex (Cotter et al., 2002) of bipolar subjects. Moreover, biochemical studies in patients with BD have demonstrated alterations in several intracellular signaling pathways that are modulated by mood stabilizers (Manji and Lenox, 2000; Bezchlibnyk and Young, 2002).

To date, the magnetic resonance spectroscopy (MRS) technique is the only direct and non-invasive method that allows the in vivo study of certain neurochemical metabolites in the human brain (Soares et al., 1996; Stanley, 2000). The use of proton MRS ($^1$HMRS) enables measurement of N-acetyl-L-aspartate (NAA), choline-containing compounds (Cho), creatine plus phosphocreatine (Cr) and myo-inositol (Ino) (Kato et al., 1998). NAA is produced in the neuronal mitochondria and is diminished in several neuropsychiatric disorders; it is considered a marker of neuronal integrity (Urenjak et al., 1993). The peak of
Cho includes many cell membrane phospholipid metabolites, including phosphocoline and glycerophosphocoline. Acetylcholine and free choline are assumed to contribute with a negligible fraction because of their relative small concentrations in the brain (Miller et al., 1996). The Cr peak contains both creatine and phosphocreatine, and once creatine is a substrate of creatine kinase, measures of Cr reflect energy metabolism status (Kato et al., 1998). *Myo*-inositol has an important function in the brain, as it is a substrate of phosphoinositide (PI) second messenger pathway (Stanley, 2002). Lithium (Li) is an uncompetitive inhibitor of the enzyme inositol monophosphatase (IMPase) at therapeutic concentrations (Ki=0.8) (Sherman et al., 1986), and this effect in the PI cycle is thought to be one of the mechanisms of action of Li (Manji and Lenox, 2000). In this regard, using $^1$HMRS, it was demonstrated that Li significantly decreased Ino levels in the frontal and anterior cingulate cortex in bipolar adults (Moore et al., 1999) and children (Davanzo et al., 2001), respectively. It was also reported that this effect was associated with treatment response (Davanzo et al., 2001; Moore et al., 1999).

Winsberg et al. (2000) studied the metabolic alterations in the prefrontal cortex of euthymic bipolar subjects, and demonstrated a significant reduction of the NAA/Cr ratios in the right and left DLPFC, compared to healthy volunteers. This finding was recently replicated in bipolar children and adolescents (Chang et al., 2003). Moreover, Cecil et al. (2002) reported a significant decrease in NAA levels in the orbitofrontal cortex of patients in manic/mixed episode. Indeed, there is a body of evidence that suggests that the DLPFC is a region probably involved in the pathophysiology of BD, and this assumption is consistent with clinical symptoms of manic patients, as the prefrontal cortex is believed to modulate volition, motivation, working memory inhibition and mood regulation (George et al., 1994; Weinberger, 1993). Therefore, it is unclear
The aim of this study was to investigate the cellular biochemistry of the DLPFC of bipolar patients in manic/mixed episode, in comparison with normal volunteers, using the $^1$H-MRS technique. We hypothesized that neural and intracellular dysfunction would be demonstrated in the DLPFC of patients in manic/mixed episode. According to our working hypothesis of intracellular dysfunction, we expected *a priori* a significant decrease of NAA and increase of *myo*inositol in the DLPFC of manic/mixed patients.
MATERIALS AND METHODS

Subjects

Ten patients (4 male, 6 female) who met the DSM-IV criteria for bipolar I disorder (BD I), manic or mixed state, and 10 comparison subjects were included in the study. All subjects gave written informed consent for participation in the study after the procedures had been fully explained in accordance with the procedures of the Hospital Espírita de Porto Alegre Ethics Committee. The demographic and clinical characteristics of patients and controls are listed in table 1. All patients were recruited from the Hospital Espírita de Porto Alegre (HEPA), and the diagnosis of BD I, manic or mixed state, was confirmed by using the Structured Clinical Interview for DSM-IV (SCID) (First et al., 1998). The healthy control subjects were also assessed by using the SCID non-patient version to rule out axis I psychiatric disorders. The mean ages (SD) of patients and controls were 36.8 ± 11.1 years and 36.1 ± 12.0 years, respectively. Nine patients and 9 controls were right-handed. Nine patients (90%) were in a manic and 1 (10%) in a mixed episode, as per DSM-IV criteria. The mean duration (SD) of illness was 12.0 ± 10.8 years. The severity of manic and depressive symptoms was assessed by the Young Mania Rating Scale (YMRS) (Young et al., 1978) and Hamilton Depression Rating Scale (HAM-D) (Hamilton, 1960), which were administered in the same day, right before the $^1$H-MRS study. The median YMRS score (range) was 35.5 (26-43) and the HAM-D score was 11 (just 1 patient fulfilled concomitant major depressive and manic episode by SCID). The severity of illness was evaluated by the Clinical Global Impression – severity – Scale (Guy, 1976). All patients were medicated with mood stabilizers and/or antipsychotics, and all but 2 patients had family history of
mood disorder or substance dependence in a first- or second-degree relative (Table 2). None of the patients had a past history of head trauma, organic mental disorder, neurological disorder, and none had a clinically significant alcohol or substance abuse in the 12 months before the study. One patient had a past history of alcohol abuse, one patient had a past history of cocaine abuse, and two patients had a past history of panic disorder. None of the controls had a past history of head trauma, organic mental disorder, neurological disorder, psychiatric disorder, or alcohol or substance abuse, and none had a family history of psychiatric disorder.

**MRI/$^{1}$HMRS Procedures**

All MRI/$^{1}$H-MRS studies were performed at the Hospital Mãe de Deus on a 1.5 Tesla General Electric Signa scanner (GE Medical Systems, Milwaukee, WI - USA) with a phased-array head coil. A sagittal T2-weighted scout series (FSE; TR: 4500ms; TE: 102ms; ET: 21; FOV: 24x24cm; MATRIX: 320x224; slice: 5mm; gap: 1.5mm) was first obtained to verify patient position and used as localizer for $^{1}$H-MRS study. The volume of interest (VOI), a 2x2x2-cm voxel (8cm$^3$) was placed in the DLPFC bilaterally, about 1.5 cm above the orbits. $^{1}$HMRS data were acquired by using a Stimulated Echo Acquisition Mode (STEAM) $^{1}$HMRS sequence at TR: 1500ms; TE: 30ms; 8 NEX (Figure 1). After the $^{1}$H-MRS data acquisition, the data analyses was performed using the Linear Combination (LC) Model Software, an operator-independent fitting routine (Provencher, 1993). The metabolite values were not corrected for cerebral spinal fluid (CSF), gray, or white matter voxel values. The following peaks were evaluated: N-acetyl-L-aspartate (NAA, 2.02 parts per million [ppm]), creatine plus phosphocreatine (Cr, 3.0 ppm), choline-containing
compounds (Cho, 3.2 ppm), myo-inositol (Ino, 3.5 ppm) (Figure 2). Only the results with fitting error less than 20% of standard deviation in the LC model were included in the statistics. The reliability of measured data was controlled by calibration of the MR scanner by the use of a phantom solution containing known chemical concentrations for spectroscopy. For statistical analysis, a one-way ANOVA was performed with the SPSS software (SPSS 10.0 for Windows, SPSS Inc. Chicago, Ill., USA), and the criterion of significance was set at p<0.05. Results were reported in absolute molar concentrations.

After the $^1$HMRS study, a fast spin echo sequence in the axial plane was used to obtain T2-weighted (FSE; TR: 4500ms; TE: 102ms; ET: 21; FOV: 24X24cm; slice: 5mm; gap: 1.5mm; MATRIX: 320 X 224), FLAIR (TR: 9000ms; TE: 120ms; TI: 2200ms; FOV: 24X24cm; slice: 5mm; gap: 1.5mm; MATRIX: 256 X 192), and volumetric T1-weighted spoiled gradient echo (SPGR) (TR: 30ms; TE: 6ms; flip angle: 45°; FOV: 20x15cm; slice: 1.5mm; MATRIX: 256x192; 124 partitions) images, in order to exclude any neuroradiological abnormality.
RESULTS

The bipolar I patients and the control group did not differ significantly in age or gender (patients = 36.8 ± 11.11; controls = 36.1 ± 12.01; df = 18; F = 0.601; p = 0.894) (Table 1). No neuroradiological abnormalities were found in the MRI images in any of the patients included in the study. There were no significant differences in absolute NAA, Cho, Cr or Ino levels, as well as NAA/Cr, Cho/Cr or Ino/Cr levels in the right or left DLPFC of bipolar patients relative to the control group (p>0.05 – Table 3). Comparing left to right DLPFC differences, we found higher absolute Ino levels in the left DLPFC, in comparison with the right DLPFC (left = 6.34 ± 2.38; right = 4.06 ± 0.82; df = 1; F = 4.999; p = 0.044) of subjects with BD. No such asymmetry was noted in the control group (p = 0.570). There were no lateralized asymmetries for absolute NAA, Cho, or Cr levels.
DISCUSSION

In the present study, we did not find any evidence of metabolic abnormality in the left or right DLPFC of medicated bipolar subjects in a manic or a mixed episode in comparison to healthy volunteers. Winsberg et al. (2000) found decreased NAA/Cr in the right and left DLPFC in 20 euthymic bipolar patients. This finding was replicated only in the right DLPFC in 15 euthymic children with familial BD (Chang et al., 2003), so it is possible that the reduction of NAA/Cr is state-dependent. In this regard, Michael et al. (2003) did not find differences in absolute NAA, Cho, or Cr levels in the left DLPFC of 8 manic subjects, and Bertolino et al. (2003) did not find differences in NAA/Cr, NAA/Cho, or Cho/Cr in the DLPFC of a mixed sample of 17 bipolar patients (7 depressed, 6 euthymic, 3 hypomanic, and 1 manic). The negative findings of the present study and some prior ones for lack of differences for the main $^1$HMRS metabolites between patients and controls may be due to the relatively small sample sizes involved and weaker statistical power, which is one limitation of our study. Nonetheless, the most likely explanation for the lack of identified differences in NAA levels is the fact that all patients in our study were on mood stabilizing medications, primarily lithium, which have been shown to change the levels of NAA and other specific $^1$HMRS metabolites, such as myo-inositol. Therefore, another limitation of the present study that may have confounded the results is the use of medications, as all patients were using mood stabilizer (9/10) and/or antipsychotic (8/10) at the time of scanning. The technical difficulty of scanning manic patients has been considered before (Moore et al., 2000), and our sample included severely manic patients (mean YMRS = 34.6 ± 5.5). In a previous study with an apparent less impaired sample
(mean YMRS = 18.8 ± 12.4), all patients were medicated as well (Cecil et al., 2002). Indeed, it has been demonstrated that lithium (Moore et al., 2000) and antipsychotics (Bertolino et al., 2001) increase NAA levels in the right frontal lobe and DLPFC, respectively, so the absence of differences in NAA levels may be due to the use of medication. The differences between our study and previous reports could be also related to methodological differences, such as mood state, medication status, image acquisition sequence, and tissue segmentation (partial volume effects).

An intriguing finding in the present study is that manic patients have higher absolute Ino levels in the left DLPFC compared to the right DLPFC. Other studies have demonstrated elevated Ino/Cr in the basal ganglia of 4 lithium-treated bipolar adults (Sharma et al., 1992), and elevated absolute Ino and Ino/Cr levels in the anterior cingulate cortex of 10 juvenile BD (7 manic and 3 in partial remission of a manic episode) (Davanzo et al., 2003). To our knowledge, this is the first report of lateralized changes in bipolar patients during manic episode using $^1$HMRS. Indeed, other investigators have reported left-to-right differences in the cortex of patients with BD (Martinot et al., 1990; Drevets et al., 1997; Deicken et al., 2001; Deicken et al., 1995). Using positron emission tomography (PET) images to measure cerebral glucose metabolism, Martinot et al. (1990) found significant left-right prefrontal asymmetry in 10 severely depressed patients (seven bipolar and three unipolar), reversible by successful treatment, whereas Drevets et al. (1997) demonstrated hypermetabolism in the left subgenual prefrontal cortex during mania, as compared to bipolar-depressed, unipolar-depressed, and controls. Using $^1$HMRS, Deicken et al. (2001) found higher left-to-right NAA in the thalamus of euthymic male BD subjects. Deicken et al. (1995) also reported higher right-to-left phosphocreatine in the frontal lobe.
of 12 euthymic unmedicated BD patients using phosphorous-31 MRS ($^{31}$PMRS), suggesting abnormal energy metabolism in BD. Taken together, these studies point out to the left hemisphere as a putative target for abnormalities in bipolar patients.

The significant increase of myoinositol in the left DLPFC in the present study may be partly explained by the severity of mania in this sample, as it has been suggested that abnormalities in the PI-signaling pathway may play a role in the pathophysiology of BD (Manji and Lenox, 2000; Bezchlibnyk and Young, 2002). Indeed, re-synthesis of PI for the maintenance of adequate PI-mediated signal transduction strongly depends on the dephosphorilation of inositol phosphates, as inositol poorly crosses the brain-blood barrier, in a reaction catalyzed by the enzyme IMPase. Studies of blood samples have demonstrated increase phosphatidylinositol 4,5-biphosphate (PIP$_2$) in platelets of bipolar patients during the manic (Brown et al., 1993) and depressive (Soares et al., 2001) states, and a significant reduction specific to platelet PIP$_2$ levels in lithium-treated euthymic patients with BD (Soares et al., 1999). One of the major targets of the PI-signaling pathway is the protein kinase C (PKC), an intracellular protein that modulates neurotransmitter release, neuronal excitability, and long-term synaptic events. In this regard, Wang and Friedman (1996) demonstrated increased PKC activity and translocation in the frontal cortex of BD subjects in a postmortem study. The same group reported increased PKC activity in platelets of BD patients during mania (Friedman et al., 1993), a finding that was not found in lithium-treated euthymic patients (Soares et al., 2000). Indeed, it has been demonstrated an inhibitory effect of lithium in PKC in animal models (Manji et al., 1993; Chen et al., 2000), an effect that has been postulated as one of the antimanic effects of lithium (Manji and Lenox, 2000). In a $^1$HMRS study, Moore et al. (1999) demonstrated that 5 days of lithium administration is sufficient to significantly decrease myoinositol in the frontal cortex of
depressed BD patients, but the clinical response occurred just 3-4 weeks after medication use, suggesting that the initial lithium-induced reduction of myoinositol may lead to a down-regulation of PKC activity and modulation of gene expression, outcomes that would ultimately account for the therapeutic effects of lithium.

In conclusion, in the present study we have demonstrated a significantly higher left-to-right ratio of DLPFC myoinositol in bipolar patients during the manic phase. This is consistent with prior findings indicating that mood disorders may involve asymmetry in brain anatomy or function (Soares and Mann, 1997). These findings provide further support for the hypothesis that myoinositol (PI-signaling pathway) may play a role in the pathophysiology of BD. No significant differences in the other $^1$HMRS metabolites were found compared to healthy controls in DLPFC. Studies in unmedicated bipolar samples that will involve a larger number of patients studied before and after mood stabilizing treatment will be required to further investigate the role of neurochemical abnormalities in prefrontal cortex in the pathophysiology of BD.
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Increased platelet membrane phosphatidylinositol-4,5-biphosphate in drug-free


Table 1. Demographic characteristics of patients and controls

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients (n=10)</th>
<th>Controls (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (SD)</td>
<td>36.8 ± 11.1</td>
<td>36.1 ± 12.0</td>
</tr>
<tr>
<td>Gender, male (%)</td>
<td>4 (40)</td>
<td>4 (40)</td>
</tr>
<tr>
<td>Right-handed (%)</td>
<td>9 (90)</td>
<td>9 (90)</td>
</tr>
<tr>
<td>Duration of illness, years</td>
<td>12.0 ± 10.8</td>
<td>N/A</td>
</tr>
<tr>
<td>Medication users (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antipsychotics</td>
<td>8 (80)</td>
<td>N/A</td>
</tr>
<tr>
<td>Mood stabilizers</td>
<td>9 (90)</td>
<td>N/A</td>
</tr>
<tr>
<td>YMRS, median (range)</td>
<td>35.5 (26-43)</td>
<td>N/A</td>
</tr>
<tr>
<td>HAM-D</td>
<td>11*</td>
<td>N/A</td>
</tr>
<tr>
<td>CGI +/- SD (range)</td>
<td>5.6 ± 0.5 (5-6)</td>
<td>N/A</td>
</tr>
<tr>
<td>Manic Episode</td>
<td>9 (90%)</td>
<td>N/A</td>
</tr>
<tr>
<td>Mixed Episode</td>
<td>1 (10%)</td>
<td>N/A</td>
</tr>
<tr>
<td>Psychotic Symptoms</td>
<td>9 (90%)</td>
<td>N/A</td>
</tr>
</tbody>
</table>

* One patient fulfilled criteria for both a major depressive and a manic episode, concurrently, by the Structured Clinical Interview for DSM-IV (SCID); N/A = not applied; YMRS = Young Mania Rating Scale; HAM-D = Hamilton Depression Rating Scale; CGI = Clinical Global Impression Scale
Table 2. Demographic and clinical characteristics of bipolar patients

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Family history of psychiatric disorders</th>
<th>Duration of illness (years)</th>
<th>Antipsychotic (AP) or mood stabilizer (MS) use</th>
<th>No. of previous episodes</th>
<th>Manic/Mixed state</th>
<th>Psychotic Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>28</td>
<td>Male</td>
<td>Yes</td>
<td>7</td>
<td>AP/MS#</td>
<td>3</td>
<td>Manic</td>
<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>37</td>
<td>Male</td>
<td>Yes</td>
<td>0.1</td>
<td>AP/MS</td>
<td>0</td>
<td>Manic</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>Male</td>
<td>Yes</td>
<td>1.3</td>
<td>AP#</td>
<td>1</td>
<td>Manic</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>59</td>
<td>Female</td>
<td>Yes</td>
<td>20</td>
<td>AP/MS</td>
<td>-</td>
<td>Manic</td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
<td>31</td>
<td>Female</td>
<td>No</td>
<td>5</td>
<td>AP/MS</td>
<td>-</td>
<td>Mixed</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>37</td>
<td>Female</td>
<td>Yes</td>
<td>20</td>
<td>AP/MS#</td>
<td>18</td>
<td>Manic</td>
<td>Yes</td>
</tr>
<tr>
<td>7</td>
<td>36</td>
<td>Male</td>
<td>Yes</td>
<td>16</td>
<td>AP/MS</td>
<td>6</td>
<td>Manic</td>
<td>Yes</td>
</tr>
<tr>
<td>8</td>
<td>48</td>
<td>Female</td>
<td>Yes</td>
<td>11</td>
<td>MS</td>
<td>7</td>
<td>Manic</td>
<td>Yes</td>
</tr>
<tr>
<td>9</td>
<td>23</td>
<td>Female</td>
<td>No</td>
<td>5</td>
<td>AP/MS</td>
<td>2</td>
<td>Manic</td>
<td>Yes</td>
</tr>
<tr>
<td>10</td>
<td>44</td>
<td>Female</td>
<td>Yes</td>
<td>35</td>
<td>MS#</td>
<td>-</td>
<td>Manic</td>
<td>Yes</td>
</tr>
</tbody>
</table>

For number of episodes, the 3 cases where the information is not listed were cases where they were unknown or too many to count.

#“Irregular” use
<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAA – R</td>
<td>7.45 ± 0.70</td>
<td>7.20 ± 0.44</td>
<td>0.42</td>
</tr>
<tr>
<td>NAA – L</td>
<td>7.83 ± 0.91</td>
<td>7.40 ± 0.76</td>
<td>0.33</td>
</tr>
<tr>
<td>Cho – R</td>
<td>1.26 ± 0.12</td>
<td>1.14 ± 0.21</td>
<td>0.21</td>
</tr>
<tr>
<td>Cho – L</td>
<td>1.31 ± 0.27</td>
<td>1.19 ± 0.22</td>
<td>0.32</td>
</tr>
<tr>
<td>Cr – R</td>
<td>5.21 ± 0.71</td>
<td>5.39 ± 0.72</td>
<td>0.62</td>
</tr>
<tr>
<td>Cr – L</td>
<td>5.83 ± 0.73</td>
<td>5.99 ± 1.38</td>
<td>0.76</td>
</tr>
<tr>
<td>Ino – R</td>
<td>4.06 ± 0.82</td>
<td>4.17 ± 0.62</td>
<td>0.75</td>
</tr>
<tr>
<td>Ino – L</td>
<td>6.34 ± 2.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.52 ± 1.63</td>
<td>0.15</td>
</tr>
<tr>
<td>NAA/Cr – R</td>
<td>1.45 ± 0.26</td>
<td>1.34 ± 0.18</td>
<td>0.37</td>
</tr>
<tr>
<td>NAA/Cr – L</td>
<td>1.38 ± 0.21</td>
<td>1.21 ± 0.18</td>
<td>0.12</td>
</tr>
<tr>
<td>Cho/Cr – R</td>
<td>0.28 ± 0.10</td>
<td>0.21</td>
<td>0.097</td>
</tr>
<tr>
<td>Cho/Cr – L</td>
<td>0.24</td>
<td>0.20</td>
<td>0.086</td>
</tr>
<tr>
<td>Ino/Cr – R</td>
<td>0.78 ± 0.15</td>
<td>0.80 ± 0.11</td>
<td>0.77</td>
</tr>
<tr>
<td>Ino/Cr – L</td>
<td>1.23 ± 0.84</td>
<td>0.94 ± 0.42</td>
<td>0.50</td>
</tr>
</tbody>
</table>

NAA = N-acetyl-L-aspartate; Cho = choline-containing compounds; Ino = myo-inositol; Cr = creatine/phosphocreatine; R = right; L = left.

<sup>a</sup>p = 0.044 Ino-R vs. Ino-L.
Figure 1 – Sagittal T2-weighted localizer scan showing the location of the DLPFC voxel

DLPFC = dorsolateral prefrontal cortex
Figure 2 – Typical HMRS spectrum

NAA = N-acetyl-L-aspartate; Cr-PCr = creatine/phosphocreatine; Cho = choline containing compounds; ml (Ino) = myo-inositol
Discussão e Conclusão da Dissertação

Não encontramos diferenças nos níveis de N-acetil-L-aspartato (NAA), compostos de colina (Cho), creatina/fosfocreatina (Cr) e myo-inositol (Ino) no córtex pré-frontal dorsolateral (CPFDL) de pacientes bipolares maníacos, em relação ao grupo controle. De fato, não há outro estudo publicado que tenha avaliado o CPFDL direito e esquerdo de bipolares em episódio maníaco. Comparando os níveis dos metabólitos do lado esquerdo e direito do CPFDL, encontramos um aumento significativo dos níveis de *myo*-inositol no CPFDL esquerdo dos pacientes em episódio maníaco/misto, sendo que esta diferença não foi observada no grupo controle. Este é o primeiro relato de diferenças significativas entre os hemisférios cerebrais em indivíduos em episódio maníaco, com o uso da espectroscopia por ressonância magnética. Nosso achado de aumento significativo do *myo*-inositol no CPFDL esquerdo pode estar associado ao alto grau de sintomas maníacos na amostra do presente estudo, uma vez que alterações na cascata de segundos-mensageiros do fosfatilinositol (PI) têm sido relatadas em indivíduos com TB.

Em conclusão, o presente estudo demonstrou, pela primeira vez, alterações *in vivo* da sinalização intracelular do segundo-mensageiro PI no CPFDL esquerdo de pacientes bipolares em episódio maníaco/misto, corroborando com o modelo de disfunção da cascata do PI na fisiopatologia do TB. Não foram demonstradas diferenças significativas dos neurometabolitos NAA, Cho, Cr e Ino entre bipolares e grupo controle. Estudos com maior controle dos procedimentos da espectroscopia por ressonância magnética e com maior amostra são necessários para melhor elucidar o papel destes metabólitos na gênese do TB. O presente estudo abre também a possibilidade de uma linha de pesquisa em modelos animais para mania, uma área que, surpreendentemente, é ainda pouco desenvolvida.
ANEXOS