

UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
INSTITUTO DE CIÊNCIAS BÁSICAS DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS:
BIOQUÍMICA

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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Porto Alegre, fevereiro de 2004.

*“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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Agradecimentos

- Ao Professor Flávio Kapczinski, meu amigo e mentor, pelo conhecimento e estímulo que me transmitiu durante toda esta jornada e pela sua compreensão nos momentos mais difíceis.
- Ao Professor Jair Soares, por todo o ensinamento em neuroimagem e pela sólida amizade construída nesse período.
- Ao Dr. Marcelo Folgieri, pela qualidade e seriedade do seu trabalho e companheirismo no decorrer desta caminhada.
- Ao Dr. Rodrigo Vieira, grande parceiro, que me incentivou a prosseguir na pós-graduação.
- Ao Mark Nicoletti, pela ajuda inestimável com a leitura dos dados da espectroscopia.
- Ao Cláudio Abs da Cruz, pelo apoio no envio dos dados para San Antonio-EUA.
- Aos funcionários do Serviço de Radiologia do Hospital Mãe de Deus, Dani, Paulinho, Ítalo, Wagner, Soraia, Gisele, entre outros.
- Aos funcionários do Serviço de Admissão do Hospital Espírita, Antônio, Cláudia, Jéferson, Jose, Nara, entre outros.
- À Manoela Fonseca, pelo apoio psicológico nos momentos mais difíceis.
- Aos pacientes e familiares que, mesmo passando por momentos delicados e estressantes, souberam valorizar a importância da pesquisa e consentiram em participar do projeto.

- À minha família, querido Tocho, pai, Tio Quico e, em especial, minha mãe, que me ensinou como ter garra e nunca desistir de um ideal, para quem eu dedico este título de Mestre.

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 (^1H -MRS) permite a mensuração de certos neurometabólitos no cérebro humano *in vivo*. Nós utilizamos a ^1H -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 (CPF DL) 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 8cm^3 localizados no CPF DL direito e esquerdo para aquisição da ^1H -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 CPF DL esquerdo em relação ao CPF DL direito ($p = 0,044$).

Conclusões: Elevação do *myo*inositol no CPF DL 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 8cm^3 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, frequentemente 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 incontidos de compras ou indiscrições 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 *myo*inositol (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 *myo*inositol, 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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Contract/grant sponsor: none financial support or conflict of interest.

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 (¹HMRS) technique allows *in vivo* measurements of certain neurometabolites, in the human brain. We used ¹HMRS 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 8cm³ voxels placed in left and right DLPFC using a short TE ¹HMRS 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 (¹H MRS) 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 ($K_i=0,8$) (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 ¹HMRS, 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 ¹H-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 ^1H -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/¹H MRS Procedures

All MRI/¹H-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 ¹H-MRS study. The volume of interest (VOI), a 2x2x2-cm voxel (8cm³) was placed in the DLPFC bilaterally, about 1.5 cm above the orbits. ¹H MRS data were acquired by using a Stimulated Echo Acquisition Mode (STEAM) ¹H MRS sequence at TR: 1500ms; TE: 30ms; 8 NEX (Figure 1). After the ¹H-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 ^1H MRS 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 ¹HMRS 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 ¹HMRS 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 ^1H MRS. 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 ^1H MRS, 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 ($^{31}\text{PMRS}$), 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 $^1\text{HMRS}$ 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 *myo*inositol 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 *myo*inositol 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 *myo*inositol (PI-signaling pathway) may play a role in the pathophysiology of BD. No significant differences in the other ¹H MRS 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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Table 1. Demographic characteristics of patients and controls

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

* 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

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

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 3. Dorsolateral prefrontal cortex metabolites (mmol/liter \pm SD)

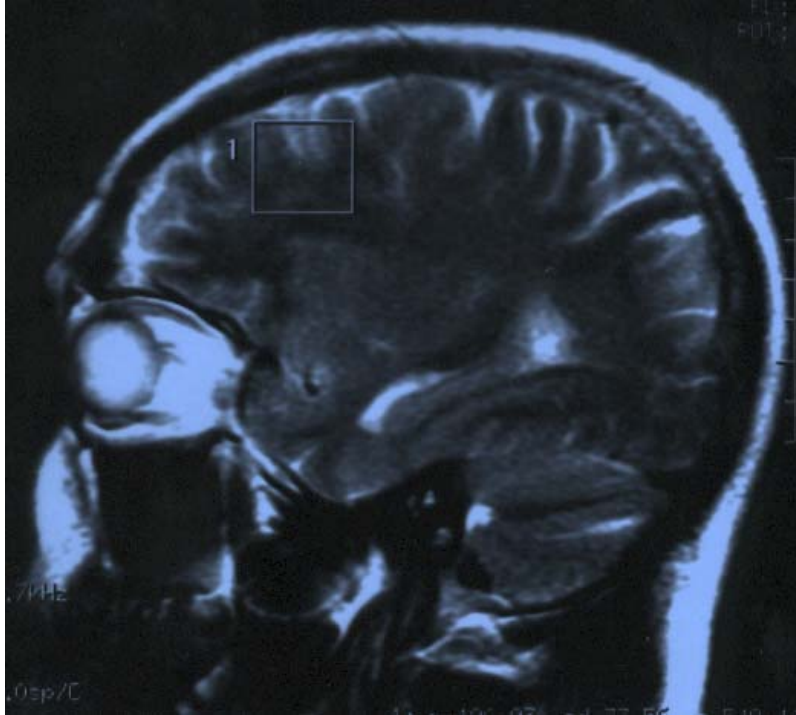
	Patients	Controls	P
NAA – R	7.45 \pm 0,70	7.20 \pm 0,44	0.42
NAA – L	7.83 \pm 0.91	7.40 \pm 0.76	0.33
Cho – R	1.26 \pm 0.12	1.14 \pm 0.21	0.21
Cho – L	1.31 \pm 0.27	1.19 \pm 0.22	0.32
Cr – R	5.21 \pm 0.71	5.39 \pm 0.72	0.62
Cr – L	5.83 \pm 0.73	5.99 \pm 1.38	0.76
Ino – R	4.06 \pm 0.82	4.17 \pm 0.62	0.75
Ino – L	6.34 \pm 2.38 ^a	4.52 \pm 1.63	0.15
NAA/Cr – R	1.45 \pm 0.26	1.34 \pm 0.18	0.37
NAA/Cr – L	1.38 \pm 0.21	1.21 \pm 0.18	0.12
Cho/Cr – R	0.28 \pm 0.10	0.21	0.097
Cho/Cr – L	0.24	0.20	0.086
Ino/Cr – R	0.78 \pm 0.15	0.80 \pm 0.11	0.77
Ino/Cr – L	1.23 \pm 0.84	0.94 \pm 0.42	0.50

NAA = N-acetyl-L-aspartate; Cho = choline-containing compounds; Ino = *myo*-inositol;

Cr = creatine/phosphocreatine; R = right; L = left.

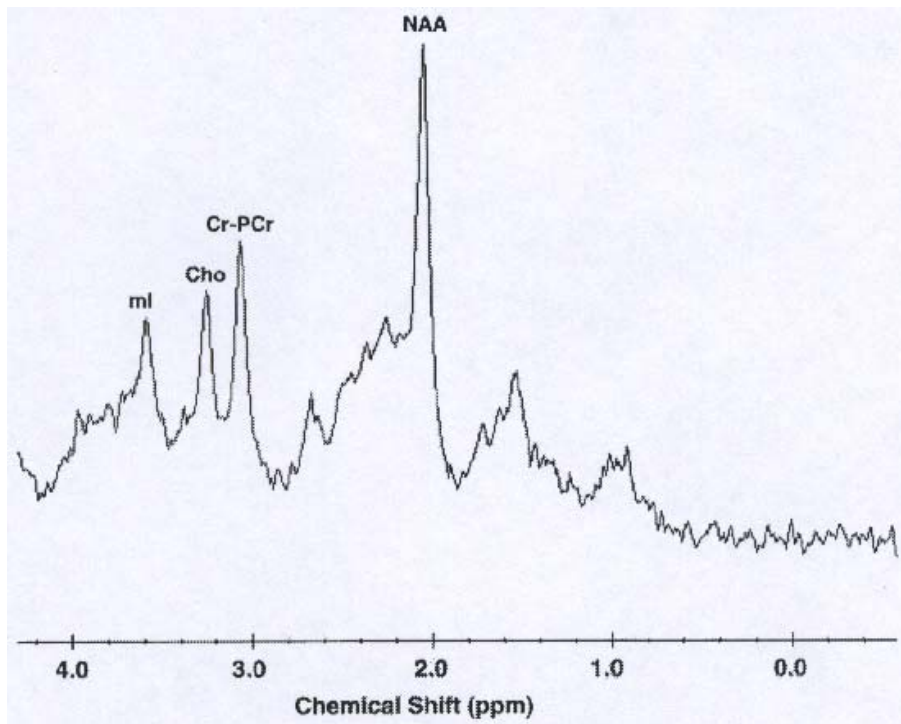
^ap = 0.044 Ino-R vs. Ino-L.

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; mI (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 (CPF DL) de pacientes bipolares maníacos, em relação ao grupo controle. De fato, não há outro estudo publicado que tenha avaliado o CPF DL direito e esquerdo de bipolares em episódio maníaco. Comparando os níveis dos metabólitos do lado esquerdo e direito do CPF DL, encontramos um aumento significativo dos níveis de *myo*inositol no CPF DL 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 CPF DL 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 fosfatitilinositol (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 CPF DL 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 neurometabólitos 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