

## Proton spectroscopy study of the left dorsolateral prefrontal cortex in pediatric depressed patients

Sheila C. Caetano<sup>a,b,c</sup>, Manoela Fonseca<sup>a,b,d</sup>, Rene L. Olvera<sup>e</sup>, Mark Nicoletti<sup>a</sup>,  
John P. Hatch<sup>a,f</sup>, Jeffrey A. Stanley<sup>g</sup>, Kristina Hunter<sup>a</sup>, Beny Lafer<sup>c</sup>,  
Steven R. Pliszka<sup>e</sup>, Jair C. Soares<sup>a,b,h,\*</sup>

<sup>a</sup> Division of Mood and Anxiety Disorders, Department of Psychiatry, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78229, USA

<sup>b</sup> Psychiatry Service, South Texas Veterans Health Care System, Audie L. Murphy Division, San Antonio, TX, USA

<sup>c</sup> Department of Psychiatry, Institute of Psychiatry, University of São Paulo School of Medicine, São Paulo, Brazil

<sup>d</sup> Psychiatry Research Unit, Federal University of Rio Grande do Sul, School of Medicine, Porto Alegre, Brazil

<sup>e</sup> Division of Child and Adolescent Psychiatry, Department of Psychiatry, The University of Texas Health Science Center at San Antonio, San Antonio, TX, USA

<sup>f</sup> Department of Orthodontics, The University of Texas Health Science Center at San Antonio, San Antonio, TX, USA

<sup>g</sup> Department of Psychiatry and Behavioral Neurosciences, Wayne State University School of Medicine, Detroit, MI, USA

<sup>h</sup> Department of Radiology, The University of Texas Health Science Center at San Antonio, San Antonio, TX, USA

Received 22 February 2005; received in revised form 28 April 2005; accepted 29 April 2005

### Abstract

The dorsolateral prefrontal cortex (DLPFC) plays an essential role in mood regulation and integration of cognitive functions that are abnormal in major depressive disorder (MDD). Few neuroimaging studies have evaluated the still maturing DLPFC in depressed children and adolescents. We conducted single voxel proton magnetic resonance spectroscopy (<sup>1</sup>H MRS) of the left DLPFC in 14 depressed children and adolescents (13.3 ± 2.3 years old, 10 males) and 22 matched healthy controls (13.6 ± 2.8 years old, 13 males). Depressed subjects had significantly lower levels of glycerophosphocholine plus phosphocholine (GPC + PC; or choline-containing compounds) and higher myo-inositol levels in the left DLPFC compared to healthy controls. In the depressed subjects, we found significant inverse correlations between glutamate levels and both duration of illness and number of episodes. In healthy controls there was a significant direct correlation between age and glutamine levels, which was not present in the patient group. Lower GPC + PC levels in pediatric MDD may reflect lower cell membrane content per volume in the DLPFC. Increased myo-inositol levels in MDD may represent a disturbed secondary messenger system. GPC + PC and myo-inositol abnormalities further demonstrate the involvement of DLPFC in pediatric MDD.

© 2005 Elsevier Ireland Ltd. All rights reserved.

**Keywords:** Choline-containing compounds; Myo-inositol; Major depression; Children; Dorsolateral prefrontal cortex; Proton spectroscopy

Major depressive disorder (MDD) has a lifetime prevalence of 15% in childhood and adolescence [19] and often presents a chronic course associated with functional impairment [32]. The investigation of the pathophysiology of MDD has led to the discovery of abnormalities in the neural structures involved in mood regulation [36]. In particular, the dorsolateral prefrontal cortex (DLPFC, Brodmann areas 9/46) plays

an essential role in mood regulation and working memory [10]. DLPFC abnormalities have been consistently found in post-mortem [7,29,40], neuroanatomical [12], neurochemical [3,16,23] and functional [2,8,18,20] studies conducted in depressed adults. Interestingly, the DLPFC is one of the last regions to mature in the human brain, probably because of its integrative role in cognitive functions [11,37]. Nevertheless, the few in vivo anatomical [24] and neurochemical [9] studies that have evaluated the DLPFC in children and adolescents suffering from MDD noted abnormalities in the left side.

\* Corresponding author. Tel.: +1 210 567 5492; fax: +1 210 567 3759.  
E-mail address: [soares@uthscsa.edu](mailto:soares@uthscsa.edu) (J.C. Soares).

Proton ( $^1\text{H}$ ) spectroscopy is a non-invasive and non-radioactive neuroimaging tool, which allows measurement of major brain chemicals such as choline-containing compounds glycerolphosphocholine + phosphocholine (GPC + PC), myo-inositol (Ino), *N*-acetyl aspartate (NAA), phosphocreatine + creatine (PCr + Cr), glutamate (Glu) and glutamine (Gln) [38].  $^1\text{H}$  spectroscopy studies of MDD adults have demonstrated multiple brain chemical abnormalities. For instance, MDD adults show lower GPC + PC/PCr + Cr ratios in the left amygdala [17] and in the basal ganglia [30], lower Glu + Gln in the cingulate [1], lower NAA/PCr + Cr ratios in the caudate, higher GPC + PC/PCr + Cr ratios in the putamen [41] compared to healthy controls. Specifically in the left DLPFC, MDD patients show higher GPC + PC/PCr + Cr and Ino/PCr + Cr ratios [16] and lower NAA/PCr + Cr ratios [23].

Post-mortem studies in adult depressed patients suggest reduced number and density of DLPFC glial cells [7,29,40]. This finding is supported by functional studies showing reduced glucose metabolism and blood flow in the DLPFC of adult depressed patients [2,8,18,20]. Interestingly, the choline-containing compounds have been associated with membrane synthesis and repair, intracellular signal transduction and the myelination process [38], which leads us to expect a reduction of GPC + PC in MDD.

We used  $^1\text{H}$  spectroscopy to compare the neurochemistry of the left DLPFC of children and adolescents suffering from MDD with that of age- and gender-matched healthy controls. Based on the previous postmortem and functional findings, we expected to find lower GPC + PC in pediatric depressed patients compared to controls. We also hypothesized that we would replicate higher Ino in pediatric depressed patients compared to healthy controls [9].

MDD subjects ( $N = 14$ ) were included if they met DSM-IV diagnostic criteria for MDD and excluded if they met lifetime diagnostic criteria for psychotic disorders, bipolar disorder, developmental disorders, substance abuse/dependence, eating disorders, Tourette' Disease, or mental retardation (demographic and clinical characteristics of pediatric MDD patients and healthy subjects are presented in Table 1).

Table 1  
Demographic and clinical characteristics of pediatric MDD patients and healthy subjects

	Pediatric MDD patients ( $N = 14$ )	Healthy controls ( $N = 22$ )
Age (years)	13.3 $\pm$ 2.3 (9.5–17.2)	13.6 $\pm$ 2.8 (8.5–17.7)
Gender (males)	10	13
Education (years)	7.1 $\pm$ 2.5	7.6 $\pm$ 2.9
SES	44.6 $\pm$ 12.7	43.4 $\pm$ 12.9
GAF	52.6 $\pm$ 6.9	87.9 $\pm$ 10.6
Age of onset (years)	10.4 $\pm$ 2.3	
Duration of illness (months)	29.13 $\pm$ 13.7	
Number of episodes	1.7 $\pm$ 1.6	
CDRS	46.6 $\pm$ 16.5	

Data are presented as mean  $\pm$  standard deviation (range).

Of the 14 depressed patients, 4 had attention deficit hyperactivity disorder (ADHD), 5 had generalized anxiety, 3 had separation anxiety, 2 had social phobia, and 1 had panic disorder. Eleven patients had a positive family history of mood disorders among their first degree-relatives (MDD ( $N = 11$ ) and bipolar disorder ( $N = 3$ )), and all patients had a positive family history of Axis I psychiatric disorders in a first degree-relative. Eight patients were free of psychotropic medication (6 were medication-naïve, 1 had last taken medication 1 year prior to the study and 1 was off medication for 2 years) and six patients were on psychotropic medication (antidepressants: sertraline ( $N = 2$ ), escitalopram ( $N = 2$ ), paroxetine ( $N = 1$ ); and/or stimulants: methylphenidate ( $N = 1$ ) and atomoxetine ( $N = 1$ )).

Healthy controls ( $N = 22$ ) were matched to the MDD subjects for age, gender and puberty status. Exclusion criteria for healthy controls were history of or current psychiatric disorder, and history of any Axis I psychiatric disorder in first-degree relatives.

Patients and controls were also required to be between 8 and 17 years old, not to have serious medical problems, and to be magnetic resonance compatible. All subjects and their parents or legal guardians were assessed using the Schedule for Affective Disorders and Schizophrenia for School-Age Children—Present and Lifetime version (K-SADS-PL) interview [15]. The severity of depression was rated using the Children's Depression Rating Scale, revised (CDRS) [27]. Puberty status was assessed through the Pubertal Development Scale–Petersen Scale [25]. Scores on the Global Assessment of Functioning Scale (GAF) were recorded. Paternal socioeconomic status (SES) was scored according to the Hollingshead Socioeconomic status [13]. This study was approved by the Institutional Review Board of The University of Texas Health Science Center at San Antonio. After the study was fully explained, written informed consent was obtained from all subjects and their parents or legal guardians.

All  $^1\text{H}$  spectroscopy scans were performed on a 1.5 T Philips Intera 8.1.1. scanner at the South Texas Veterans Health Care System (Audie Murphy Division). The MRI studies were performed with a T1-weighted fast field echo sequence (3D T1-FFE), with repetition time (TR) of 25 ms, echo time (TE) of 5 ms, field of view (FOV) of 240 mm  $\times$  220 mm, slice thickness of 1.0 mm, gap = 0, number of excitations (NEX) of 2, and matrix size of 256  $\times$  192.

A single-voxel  $^1\text{H}$  spectroscopy approach [PRESS sequence (point-resolved spectroscopy), TR of 6 s, TE of 30 ms, bandwidth of 4 kHz, 4096 complex data points] was used to localize a 2 cm  $\times$  2 cm  $\times$  2 cm voxel placed in the left DLPFC. Based on high-resolution anatomical images, the superior frontal sulcus, the lateral fissure, and the genu of corpus callosum were used as anatomical landmarks for placement of the MRS voxel [14] (Fig. 1). We also collected water-suppressed spectra for absolute quantification. The LC Model software package was used to quantify the  $^1\text{H}$  metabolites which include: NAA, Gln, Glu, Ino, GPC + PC, PCr + Cr, taurine, alanine, aspartate,  $\gamma$ -amino-butyric acid (GABA),

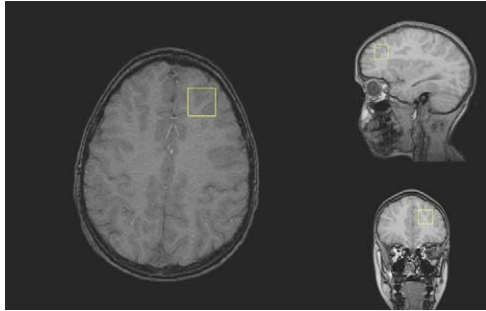


Fig. 1. Anatomical landmarks for placement of the left MRS voxel.

glucose, scyllo-inositol and NAAG as well as lipid resonances and macromolecule resonances [33]. However, only results of the more reliable metabolites (NAA, PCr+Cr, GPC+PC, Ino, Gln, and Glu) were used in the analysis [28].

Statistical analyses were performed in SPSS, version 12, for Windows software (SPSS Inc., Chicago, IL). We adopted a two-tailed significance level of  $p < 0.05$ . For spectroscopy measures that followed normal distribution, we conducted analysis of covariance (ANCOVA) with age and gender as covariates. For spectroscopy measures that did not follow a normal distribution, we conducted a non-parametric median test for independent samples. Pearson's correlation coefficients were used to examine linear association between chemical measures and age, and Spearman's correlation coefficients were used for clinical variables that did not follow a normal distribution (duration of illness, number of depressive episodes, CDRS). Two of the  $^1\text{H}$  MRS measures, GPC+PC and Ino, were considered primary based on our a priori predictions. Other measures were analyzed on an exploratory basis.

Depressed patients and healthy controls did not differ significantly in terms of age ( $t = 0.79$ , d.f. = 34,  $p = 0.28$ ), gender (Fisher's exact test,  $p = 0.50$ ), puberty degree ( $U = 139.000$ , d.f. =  $-0.51$ ,  $p = 0.64$ ) or education ( $t = 0.48$ , d.f. = 34,  $p = 0.64$ ; Table 1).

Means and standard deviations of all metabolite levels for depressed patients and healthy controls are displayed in Table 2. Our prediction of lower GPC+PC levels in MDD subjects was confirmed. Depressed patients had significantly lower levels of GPC+PC ( $p = 0.002$ , ANCOVA with age and gender as covariates). This analysis was repeated, including

only medication-free depressed patients and healthy controls, and we confirmed the lower GPC+PC levels in depressed patients ( $F = 6.62$ , d.f. = 1/26,  $p = 0.016$ ). Our prediction of higher Ino levels in depressed subjects also was confirmed. Myo-inositol levels ( $\chi^2 = 10.11$ ,  $p = 0.001$ , median test; median = 3.78, quartiles: 3.64–3.86 versus median = 3.31, quartiles: 3.05–3.60) were significantly higher in depressed patients compared to healthy controls. No significant differences were found between depressed patients and healthy controls in any of the other chemicals in Table 2.

We repeated our analysis excluding the MDD patients with positive family history of bipolar disorder. We obtained similar results of lower GPC+PC levels ( $F = 8.03$ , d.f. = 1/29,  $p = 0.08$ ) and higher Ino levels ( $\chi^2 = 7.34$ ,  $p = 0.019$ ) in MDD patients with positive family history of MDD compared to healthy controls.

Healthy controls demonstrated a significant direct linear association between age and Gln concentrations ( $r = 0.62$ ,  $p = 0.002$ ), and a non-significant trend for age and NAA levels ( $r = 0.41$ ,  $p = 0.056$ ). In depressed patients, there were no significant correlations between age and neurochemical levels.

In the MDD patients, we found a significant inverse correlation between Glu levels and duration of illness ( $\rho = -0.63$ ,  $p = 0.022$ ). No other significant correlations were found.

Predictions of lower GPC+PC levels within the left DLPFC of MDD children and adolescents compared to healthy controls were confirmed. These findings conflict with previous study, which reported higher GPC+PC levels in the left DLPFC of MDD adolescents compared to healthy controls [9]. However, that MDD sample had higher CDRS scores (62.8 versus 46.6) and a shorter mean duration of illness (22 versus 29 months) than ours. Our GPC+PC findings are consistent with a report of an inverse correlation between GPC+PC/PCr+Cr ratios in the amygdala and depression severity (measured by Beck Depression Inventory) in MDD children and adolescents [17]. Another previous study reported lower GPC+PC levels in bipolar adults who were manic or in mixed state compared to healthy controls [4]. However, MDD adolescents had higher GPC+PC/Cr ratios in the orbitofrontal cortex [39] and no alterations in GPC+PC levels in the thalamus [35] compared to healthy controls. Contradictory findings in basal ganglia were also reported. One study showed lower GPC+PC/PCr+Cr ratios in MDD

Table 2  
Mean ( $\pm 1$  S.D.) metabolite absolute values of the left DLPFC for depressed patients and healthy controls

Absolute metabolite level (mM)	Depressed subjects ( $N = 14$ )	Healthy controls ( $N = 22$ )	Statistics
GPC+PC	1.14 $\pm$ 0.14	1.35 $\pm$ 0.20	$F = 11.72$ , $p = 0.002^a$
Ino	3.71 $\pm$ 0.25	3.43 $\pm$ 0.54	$\chi^2 = 10.11$ , $p = 0.001^b$
NAA	6.91 $\pm$ 0.44	7.16 $\pm$ 0.58	$F = 1.99$ , $p = 0.17^a$
PCr-Cr	5.10 $\pm$ 0.36	5.07 $\pm$ 0.53	$\chi^2 = 0.47$ , $p = 0.73^b$
Gln	2.27 $\pm$ 0.76	2.55 $\pm$ 0.97	$F = 0.83$ , $p = 0.37^a$
Glu	5.75 $\pm$ 0.54	5.76 $\pm$ 0.87	$F = 0.01$ , $p = 0.96^a$

<sup>a</sup> ANCOVA with age and gender as covariates, d.f. = 1/34.

<sup>b</sup> Median test.

adults, which were more pronounced in selective serotonin receptor inhibitors–responders [30], but another study demonstrated higher GPC + PC/PCr + Cr ratios in elderly depressed patients, which decreased after antidepressant treatment [6]. We repeated our analysis, including only medication-free depressed patients; and confirmed the lower GPC + PC levels. Hence, it is unlikely that our findings reflect only medication effects.

Bearing in mind that GPC + PC is associated with membrane turn-over and the myelination process, our finding of lower GPC + PC levels could be interpreted as evidence of diminished cell growth or myelination in MDD compared to healthy controls. These interpretations are consistent with postmortem studies of DLPFC showing reduced glial number and density in adults with MDD [7,29,40]. Moreover, the packing density of glial fibrillary acidic protein (GFAP) immunoreactive astrocytes is reduced in adult depressed patients and also directly correlated with age of depression onset [34]. The functional neuroimaging studies showing reduced blood flow and glucose metabolism in DLPFC [2,8,18,20] of adults with MDD further corroborate lower GPC + PC in MDD.

The other neurochemical measured was Ino, which is responsible for second and third messenger intracellular activity. We found higher Ino levels in the left DLPFC of pediatric MDD patients, replicating previous findings in depressed adults [16] and in mood disorder adolescents with at least one parent with bipolar disorder [5].

No abnormal NAA levels were observed in the left DLPFC of depressed adolescents, which is in agreement with a previous report [9]. NAA/PCr + Cr ratios were lower in elderly depressed subjects with late onset (above 50 years old) depression but not those with early onset depression or healthy controls. Furthermore, NAA/PCr + Cr ratios also were associated with deep white matter lesions [23], possibly due to the role of NAA in the maintenance of myelin. We found a trend towards a direct correlation between age and NAA in healthy controls, which could be interpreted as evidence of neuronal maturation, as NAA is predominately present in mature oligodendrocytes. No such trend was found in MDD patients.

Even though we did not find lower levels of Glu neurotransmitter or its precursor Gln in the left DLPFC of depressed patients, we noted a direct correlation between age and Gln in healthy children and adolescents and an inverse correlation between Glu and duration of illness in MDD patients. Taken our findings together, we could speculate that older depressed patients with longer duration of illness would have lower Gln concentrations compared to healthy controls. In depressed adolescents, there is a direct correlation between Glu + Gln concentration in the cingulate and severity of functional impairment, which we did not find in the DLPFC as assessed by GAF [22]. Lower Glu + Gln concentrations have been reported in the cingulate of depressed adults [1] and adolescents [22,31], but not in the left DLPFC of depressed adolescents [9] and adults

[16,23]. Moreover, Glu + Gln concentrations in the DLPFC [21] and in the cingulate [26] of depressed adult patients increase to normal levels after ECT treatment.

Limitations of this study are its relatively small sample size and the fact that 6 pediatric patients were on psychotropic medication. Another possible confounder is the presence of comorbidities such as anxiety disorders and ADHD. The latter, however, are highly prevalent and their presence may be part of the clinical presentation and pathophysiology of pediatric MDD. Furthermore, all previous studies conducted in children and adolescents with MDD also included anxiety disorder comorbidities [9,22,31]. Another concern is that most MDD subjects were depressed at scan time. In this sense, our findings are probably related to mood state. Moreover, in a functional study, MDD adults in remission presented a normalization of the DLPFC metabolism indicating that the hypometabolism was a state abnormality [20].

Possible limitations of our MRS methods are the use of absolute concentrations that usually include a certain margin of error, due to calculations made relative to unsuppressed water and standard brain concentrations of such. An advantage is the sensitivity to detect a significant difference in Glu and Gln. Normal variation in brain morphology may have limited the consistency of voxel placement. We studied only the left hemisphere because the prior neuroimaging studies reported abnormalities only in the left DLPFC.

In conclusion, lower GPC + PC levels in pediatric MDD may be related to lower membrane turn-over and subsequently lower glial cell numbers in the left DLPFC. Higher Ino levels in MDD may indicate abnormalities in the phosphoinositol second messenger system. Altogether, GPC + PC and Ino abnormalities further support the involvement of impairment in the left DLPFC in pediatric MDD. Future studies in high risk MDD subjects are needed, as they may contribute to the understanding of the role of DLPFC in the pathophysiology of this disorder.

## Acknowledgments

This work was partly supported by MH01736, MH068662, UTHSCSA GCRC (M01-RR-01346), the Krus Endowed Chair in Psychiatry (UTHSCSA), the Veterans Administration, and Capes Foundation (Brazil).

## References

- [1] D.P. Auer, B. Putz, E. Kraft, B. Lipinski, J. Schill, F. Holsboer, Reduced glutamate in the anterior cingulate cortex in depression: an in vivo proton magnetic resonance spectroscopy study, *Biol. Psychiatry* 47 (2000) 305–313.
- [2] M. Beauregard, J. Levesque, P. Bourgouin, Neural correlates of conscious self-regulation of emotion, *J. Neurosci.* 21 (2001) RC165.
- [3] N. Binesh, A. Kumar, S. Hwang, J. Mintz, M.A. Thomas, Neurochemistry of late-life major depression: a pilot two-dimensional MR spectroscopic study, *J. Magn. Reson. Imaging* 20 (2004) 1039–1045.

- [4] K.M. Cecil, M.P. DelBello, R. Morey, S.M. Strakowski, Frontal lobe differences in bipolar disorder as determined by proton MR spectroscopy, *Bipolar Disord.* 4 (2002) 357–365.
- [5] K.M. Cecil, M.P. DelBello, M.C. Sellars, S.M. Strakowski, Proton magnetic resonance spectroscopy of the frontal lobe and cerebellar vermis in children with a mood disorder and a familial risk for bipolar disorders, *J. Child. Adolesc. Psychopharmacol.* 13 (2003) 545–555.
- [6] H.C. Charles, F. Lazeyras, K.R. Krishnan, O.B. Boyko, M. Payne, D. Moore, Brain choline in depression: in vivo detection of potential pharmacodynamic effects of antidepressant therapy using hydrogen localized spectroscopy, *Prog. Neuropsychopharmacol. Biol. Psychiatry* 18 (1994) 1121–1127.
- [7] D. Cotter, S. Landau, C. Beasley, R. Stevenson, G. Chana, L. MacMillan, I. Everall, The density and spatial distribution of GABAergic neurons, labelled using calcium binding proteins, in the anterior cingulate cortex in major depressive disorder, bipolar disorder, and schizophrenia, *Biol. Psychiatry* 51 (2002) 377–386.
- [8] W.C. Drevets, J.L. Price, J.R. Simpson Jr., R.D. Todd, T. Reich, M. Vannier, M.E. Raichle, Subgenual prefrontal cortex abnormalities in mood disorders, *Nature* 386 (1997) 824–827.
- [9] T.R. Farchione, G.J. Moore, D.R. Rosenberg, Proton magnetic resonance spectroscopic imaging in pediatric major depression, *Biol. Psychiatry* 52 (2002) 86–92.
- [10] P. Fossati, A.M. Ergis, J.F. Allilaire, Executive functioning in unipolar depression: a review, *Encephale* 28 (2002) 97–107.
- [11] J.N. Giedd, J. Blumenthal, N.O. Jeffries, F.X. Castellanos, H. Liu, A. Zijdenbos, T. Paus, A.C. Evans, J.L. Rapoport, Brain development during childhood and adolescence: a longitudinal MRI study, *Nat. Neurosci.* 2 (1999) 861–863.
- [12] R.S. Hastings, R.V. Parsey, M.A. Oquendo, V. Arango, J.J. Mann, Volumetric analysis of the prefrontal cortex, amygdala, and hippocampus in major depression, *Neuropsychopharmacology* 29 (2004) 952–959.
- [13] A. Hollingshead, Two-Factor Index of Social Position, Department of Sociology, Yale University, New Haven, CT, 1965.
- [14] G.D. Jackson, J.S. Duncan, MRI Anatomy: A New Angle on the Brain, Churchill Livingstone, 1996.
- [15] J. Kaufman, B. Birmaher, D. Brent, U. Rao, N. Ryan, Diagnostic Interview—Kiddie—Sads—Present and Lifetime Version (K-SADS-PL), 1996.
- [16] A. Kumar, A. Thomas, H. Lavretsky, K. Yue, A. Huda, J. Curran, T. Venkatraman, L. Estanol, J. Mintz, M. Mega, A. Toga, Frontal white matter biochemical abnormalities in late-life major depression detected with proton magnetic resonance spectroscopy, *Am. J. Psychiatry* 159 (2002) 630–636.
- [17] V. Kusumakar, F.P. MacMaster, L. Gates, S.J. Sparkes, S.C. Khan, Left medial temporal cytosolic choline in early onset depression, *Can. J. Psychiatry* 46 (2001) 959–964.
- [18] J. Levesque, F. Eugene, Y. Joanne, V. Paquette, B. Mensour, G. Beaudoin, J.M. Leroux, P. Bourgouin, M. Beaugard, Neural circuitry underlying voluntary suppression of sadness, *Biol. Psychiatry* 53 (2003) 502–510.
- [19] P.M. Lewinsohn, H. Hops, R.E. Roberts, J.R. Seeley, J.A. Andrews, Adolescent psychopathology: I. Prevalence and incidence of depression and other DSM-III-R disorders in high school students, *J. Abnorm. Psychol.* 102 (1993) 133–144.
- [20] H.S. Mayberg, M. Liotti, S.K. Brannan, S. McGinnis, R.K. Mahurin, P.A. Jerabek, J.A. Silva, J.L. Tekell, C.C. Martin, J.L. Lancaster, P.T. Fox, Reciprocal limbic–cortical function and negative mood: converging PET findings in depression and normal sadness, *Am. J. Psychiatry* 156 (1999) 675–682.
- [21] N. Michael, A. Erfurth, P. Ohrmann, V. Arolt, W. Heindel, B. Pfeleiderer, Metabolic changes within the left dorsolateral prefrontal cortex occurring with electroconvulsive therapy in patients with treatment resistant unipolar depression, *Psychol. Med.* 33 (2003) 1277–1284.
- [22] Y. Mirza, J. Tang, A. Russell, S.P. Banerjee, R. Bhandari, J. Ivey, M. Rose, G.J. Moore, D.R. Rosenberg, Reduced anterior cingulate cortex glutamatergic concentrations in childhood major depression, *J. Am. Acad. Child Adolesc. Psychiatry* 43 (2004) 341–348.
- [23] T. Murata, H. Kimura, M. Omori, H. Kado, H. Kosaka, T. Iidaka, H. Itoh, Y. Wada, MRI white matter hyperintensities, (1)H-MR spectroscopy and cognitive function in geriatric depression: a comparison of early- and late-onset cases, *Int. J. Geriatr. Psychiatry* 16 (2001) 1129–1135.
- [24] C.L. Nolan, G.J. Moore, R. Madden, T. Farchione, M. Bartoi, E. Lorch, C.M. Stewart, D.R. Rosenberg, Prefrontal cortical volume in childhood-onset major depression: preliminary findings, *Arch. Gen. Psychiatry* 59 (2002) 173–179.
- [25] A.C. Pertersen, L. Crockett, M. Richards, A. Boxer, A self-report measure of pubertal status: reliability, validity, and initial norms, *J. Youth Adolesc.* 17 (1988) 117–133.
- [26] B. Pfeleiderer, N. Michael, A. Erfurth, P. Ohrmann, U. Hohmann, M. Wolgast, M. Fiebich, V. Arolt, W. Heindel, Effective electroconvulsive therapy reverses glutamate/glutamine deficit in the left anterior cingulum of unipolar depressed patients, *Psychiatry Res.* 122 (2003) 185–192.
- [27] E.O. Poznanski, S.C. Cook, B.J. Carroll, A depression rating scale for children, *Pediatrics* 64 (1979) 442–450.
- [28] S.W. Provencher, Estimation of metabolite concentrations from localized in vivo proton NMR spectra, *Magn. Reson. Med.* 30 (1993) 672–679.
- [29] G. Rajkowska, J.J. Miguel-Hidalgo, J. Wei, G. Dilley, S.D. Pittman, H.Y. Meltzer, J.C. Overholser, B.L. Roth, C.A. Stockmeier, Morphometric evidence for neuronal and glial prefrontal cell pathology in major depression, *Biol. Psychiatry* 45 (1999) 1085–1098.
- [30] P.F. Renshaw, B. Lafer, S.M. Babb, M. Fava, A.L. Stoll, J.D. Christensen, C.M. Moore, D.A. Yurgelun-Todd, C.M. Bonello, S.S. Pillay, A.J. Rothschild, A.A. Nierenberg, J.F. Rosenbaum, B.M. Cohen, Basal ganglia choline levels in depression and response to fluoxetine treatment: an in vivo proton magnetic resonance spectroscopy study, *Biol. Psychiatry* 41 (1997) 837–843.
- [31] D.R. Rosenberg, Y. Mirza, A. Russell, J. Tang, J.M. Smith, S.P. Banerjee, R. Bhandari, M. Rose, J. Ivey, C. Boyd, G.J. Moore, Reduced anterior cingulate glutamatergic concentrations in childhood OCD and major depression versus healthy controls, *J. Am. Acad. Child Adolesc. Psychiatry* 43 (2004) 1146–1153.
- [32] N.D. Ryan, Child and adolescent depression: short-term treatment effectiveness and long-term opportunities, *Int. J. Methods Psychiatr. Res.* 12 (2003) 44–53.
- [33] U. Seeger, U. Klose, I. Mader, W. Grodd, T. Nagele, Parameterized evaluation of macromolecules and lipids in proton MR spectroscopy of brain diseases, *Magn. Reson. Med.* 49 (2003) 19–28.
- [34] X. Si, J.J. Miguel-Hidalgo, G. O'Dwyer, C.A. Stockmeier, G. Rajkowska, Age-dependent reductions in the level of glial fibrillary acidic protein in the prefrontal cortex in major depression, *Neuropsychopharmacology* 29 (2004) 2088–2096.
- [35] E.A. Smith, A. Russell, E. Lorch, S.P. Banerjee, M. Rose, J. Ivey, R. Bhandari, G.J. Moore, D.R. Rosenberg, Increased medial thalamic choline found in pediatric patients with obsessive–compulsive disorder versus major depression or healthy control subjects: a magnetic resonance spectroscopy study, *Biol. Psychiatry* 54 (2003) 1399–1405.
- [36] J.C. Soares, J.J. Mann, The anatomy of mood disorders—review of structural neuroimaging studies, *J. Psychiatr. Res.* 41 (1997) 86–106.
- [37] E.R. Sowell, P.M. Thompson, C.J. Holmes, T.L. Jernigan, A.W. Toga, In vivo evidence for post-adolescent brain maturation in frontal and striatal regions, *Nat. Neurosci.* 2 (1999) 859–861.
- [38] J.A. Stanley, In vivo magnetic resonance spectroscopy and its application to neuropsychiatric disorders, *Can. J. Psychiatry* 47 (2002) 315–326.

- [39] R.J. Steingard, D.A. Yurgelun-Todd, J. Hennen, J.C. Moore, C.M. Moore, K. Vakili, A.D. Young, A. Katic, W.R. Beardslee, P.F. Renshaw, Increased orbitofrontal cortex levels of choline in depressed adolescents as detected by in vivo proton magnetic resonance spectroscopy, *Biol. Psychiatry* 48 (2000) 1053–1061.
- [40] N.A. Uranova, V.M. Vostrikov, D.D. Orlovskaya, V.I. Rachmanova, Oligodendroglial density in the prefrontal cortex in schizophrenia and mood disorders: a study from the Stanley Neuropathology Consortium, *Schizophr. Res.* 67 (2004) 269–275.
- [41] M. Vythilingam, H.C. Charles, L.A. Tupler, T. Blitchington, L. Kelly, K.R. Krishnan, Focal and lateralized subcortical abnormalities in unipolar major depressive disorder: an automated multivoxel proton magnetic resonance spectroscopy study, *Biol. Psychiatry* 54 (2003) 744–750.