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# Metabolic abnormalities in fronto-striatal-thalamic white matter tracts in schizophrenia

Clare L. Beasley [a,*], Andrew J. Dwork [b,c,d], Gorazd Rosoklija [b,c,e], J. John Mann [b,c], Branislav Mancevski [b,c], Zlatko Jakovski [f], Natasa Davceva [f], Andrew R. Tait [g], Suzana K. Straus [g], William G. Honer [a]

[a] Department of Psychiatry, University of British Columbia, Vancouver, BC, Canada
[b] Department of Molecular Imaging and Neuropathology, New York State Psychiatric Institute, New York, NY, USA
[c] Department of Psychiatry, College of Physicians and Surgeons of Columbia University, New York, NY, USA
[d] Department of Pathology, College of Physicians and Surgeons of Columbia University, New York, NY, USA
[e] Macedonian Academy of Sciences and Arts, University "SS. Cyril and Methodius," Skopje, Macedonia
[f] Institute for Forensic Medicine, University "SS. Cyril and Methodius," Skopje, Macedonia
[g] Department of Chemistry, University of British Columbia, Vancouver, BC, Canada

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ABSTRACT

The anterior limb of the internal capsule (ALIC) is the major white matter tract providing reciprocal connections between the frontal cortex, striatum and thalamus. Mounting evidence suggests that this tract may be affected in schizophrenia, with brain imaging studies reporting reductions in white matter volume and density, changes in fractional anisotropy and reduced asymmetry. However, the molecular correlates of these deficits are currently unknown. The aim of this study was to identify alterations in protein and metabolite levels in the ALIC in schizophrenia. Samples were obtained post-mortem from individuals with schizophrenia ($n=15$) and non-psychiatric controls ($n=13$). Immunoreactivity for the myelin-associated protein myelin basic protein (MBP), and the axonal-associated proteins phosphorylated neurofilament and SNAP-25 was measured by enzyme-linked immunoadsorbent assay (ELISA). Metabolite concentrations were quantified by proton nuclear magnetic resonance ($^1$H NMR) spectroscopy. Levels of myelin- or axonal-associated proteins did not differ between groups. Overall differences in metabolite concentrations were observed between the two groups (MANOVA $F=2.685$, $p=0.036$), with post-hoc tests revealing lower lactate (19%) and alanine (24%) levels in the schizophrenia group relative to controls. Observed changes in lactate and alanine levels indicate metabolic abnormalities within the ALIC in schizophrenia.


## 1. Introduction

Alterations in fronto-striato-thalamic circuitry are hypothesised to be integral to the pathophysiology of schizophrenia (Andreasen et al., 1994; Robbins, 1990). Disruption of one or more cortical, striatal, thalamic or white matter components could result in dysfunction of these circuits. Each of these brain regions has been implicated in schizophrenia. For instance, structural imaging studies provide some evidence for thinning of the prefrontal cortex (Kuperberg et al., 2003, Narr et al., 2005) and similar volume deficits have been noted in the dorsomedial thalamus (Kemether et al., 2003; Shimizu et al., 2008). Striatal volumes are labile and are often affected by antipsychotic drug administration (Chakos et al., 1994; Lang et al., 2004). Post-mortem investigations have identified abnormalities at the cellular level in these brain regions, including deficits in neuronal and glial subtypes and decreased synaptic density (reviewed in Harrison, 1999; Harrison and Weinberger, 2005).

* Corresponding author. Department of Psychiatry, University of British Columbia, BC Mental Health and Addictions Research Institute, #A3-115 938 West 28th Ave., Vancouver, BC, Canada V5Z 4H4. Tel.: +1 604 875 2000x4723; fax: +1 604 875 3871.
*E-mail address:* cbeasley@interchange.ubc.ca (C.L. Beasley).

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Brain imaging studies have also offered evidence for abnormalities of the anterior limb of the internal capsule (ALIC), the white matter tract that connects the prefrontal cortex, striatum and thalamus, in schizophrenia. The volume of this tract is smaller (Lang et al., 2006; Zhou et al., 2003), its density reduced (Hulshoff Pol et al., 2004; McIntosh et al., 2005) and its length shorter (Buchsbaum et al., 2006), while reported alterations in fractional anisotropy (Kubicki et al., 2005; Mitelman et al., 2007) suggest an abnormality in the organization of fibres.

The cellular and molecular correlates of white matter deficits reported in schizophrenia are not yet fully resolved. Abnormalities of oligodendrocytes and myelin could represent a potential source of disrupted neural connectivity. However, while there are reports of decreased oligodendrocyte numbers, as well as reduced expression of myelin-related genes and proteins in frontal regions, most previous studies have investigated cortical grey matter. Lower oligodendrocyte density has been reported in frontal white matter in a small sample of elderly subjects with schizophrenia (Hof et al., 2003), but myelin protein expression has not yet been quantified in specific white matter tracts. Alterations in the ALIC could also reflect axonal pathology. Although axon density has not been assessed in this brain region, decreased fibre density has been reported in the anterior commissure (Highley et al., 1999a) and corpus callosum (Highley et al., 1999b) in female patients. However, a further study failed to find any difference in the total length of myelinated fibres in frontal white matter (Marner and Pakkenberg, 2003). Metabolic abnormalities have also been reported in white matter in schizophrenia. Reduced N-acetyl aspartate (NAA) levels in patients, identified using proton magnetic resonance spectroscopy (Lim et al., 1998), may indicate altered axonal integrity. Spectroscopy can also be performed on post-mortem samples, with greater resolution compared with living patients. This technique is novel, and we know of only one previous investigation of the metabolome in white matter in schizophrenia (Prabakaran et al., 2004). This study reported decreased myoinositol, phosphocholine and acetate, along with increased glutamate and lactate concentrations in schizophrenia, possibly reflective of metabolic changes.

The anterior limb of the internal capsule is comprised of two major fibre systems; the anterior thalamic peduncle, which connects the anterior and dorsomedial thalamic nuclei and the prefrontal cortex, and the frontopontine tract, which connects the frontal cortex with the pons (Axer et al., 1999; Axer and Keyserlingk, 2000). Fronto-striato-thalamic circuitry is organized anatomically and functionally into a number of 'loops', which include dorsal and ventral components (Alexander et al., 1986; Chudasama and Robbins, 2006). The dorsal circuit comprises the dorsolateral prefrontal cortex, the dorsolateral caudate and the parvicellular division of the dorsomedial thalamic nucleus, projecting through the dorsal portion of the ALIC, while the ventral circuit comprises the orbitofrontal cortex, the ventromedial caudate and the magnocellular part of the dorsomedial nucleus, projecting via the ventral portion of the ALIC (Alexander et al., 1986; Tekin and Cummings, 2002).

The objectives of the present study were to investigate the ALIC as a marker of fronto-striatal-thalamic connectivity in schizophrenia. Fronto-striatal-thalamic pathways have received much attention, through studies of normal function in cognitive activity, and dysfunction in schizophrenia and in neurological disorders. Dysfunction related to the dorsal circuit results in impaired executive functions, such as working memory, while dysfunction of the ventral circuit impairs reversal learning and disrupts behavioural responses (Clark et al., 2004; Robbins, 2007; Tekin and Cummings, 2002). In this study we assessed several measures of structural and functional integrity, including myelin-, axonal- and glial-associated proteins and cellular metabolites, in dorsal and ventral regions of the ALIC to identify alterations in schizophrenia.

## 2. Materials and methods

### 2.1. Subjects

Frozen samples consisting of ventral and dorsal portions of the ALIC were obtained from the Macedonian/New York State Psychiatric Institute Brain Collection. Studies were approved by the Clinical Research Ethics Board of the University of British Columbia and by the Institutional Review Boards of the New York State Psychiatric Institute and the College of Physicians and Surgeons, Columbia University. The sample consisted of post-mortem brain tissue from 28 subjects: 15 individuals with schizophrenia, including two with schizoaffective disorder (DSM-IV criteria), and 13 non-psychiatric controls. All subjects died from acute causes, and no deaths were due to suicide. Detailed case summaries are provided in Table 1. Brain specimens were tested for the presence of psychoactive drugs using capillary gas chromatography, with confirmation by mass spectrometry. Tissue was obtained from the right hemisphere in each case.

**Table 1**
Demographic and clinical variables in control ($n=13$) and schizophrenia ($n=15$) subjects.

| | Control | | Schizophrenia | |
|---|---|---|---|---|
| | Mean +/− SD ($n$) | Range | Mean +/− SD ($n$) | Range |
| Age (years) | 51.4 +/− 18.8(13) | 26–81 | 53.6 +/− 12.1 (1 5) | 33–77 |
| Sex (M/F) | 10/3 | | 9/6 | |
| Postmortem time (hours) | 16.6 +/− 7.1 (13) | 6–33 | 8.8 +/− 3.2 (15) | 6–15 |
| Duration of storage (weeks) | 196 +/− 77 | 68–256 | 316 +/− 74 (15) | 218–417 |
| pH | 6.16 +/− 0.33 | 5.44–6.77 | 6.35 +/− 0.27 | 5.9–6.9 |
| Cause of death | Motor vehicle accident (5)<br>Cardiac (4)<br>Homicide (3)<br>Accidental (1) | | Cardiac (7)<br>Gastrointestinal (3)<br>Pulmonary (3)<br>Accidental (2) | |

### 2.2. Tissue preparation

Coronal slabs of the right hemisphere were cut at autopsy and frozen at −80 °C. Samples of the dorsal and ventral portions of the ALIC were sub-dissected at a standardized level without letting the tissue thaw. The samples were weighed and homogenized in 10 volumes of ice-cold buffered saline.

### 2.3. Enzyme-linked immunoadsorbent assay

Levels of protein markers associated with myelin (myelin basic protein [MBP]), axons (the cytoskeletal protein phosphorylated neurofilament [PNF], and the synaptic protein SNAP-25) and astrocytes (glial fibrillary acidic protein [GFAP]) were analyzed in both dorsal and ventral regions separately. Immunoreactivity for MBP (SMI-94, 1:500, Sternberger Monoclonals), PNF (SMI-34, 1:200, Sternberger Monoclonals), SNAP-25 (SP12, 1:10) and GFAP (SMI-22, 1:200, Sternberger Monoclonals) was determined by ELISA, as previously described (Beasley et al., 2005; Flynn et al., 2003; Honer et al., 1999). Briefly, homogenates were diluted to a starting concentration of 15, 30, 60 and 30 μg protein/ml for MBP, PNF, SNAP-25 and GFAP respectively. Duplicate samples were serially diluted over a 128-fold range and dried onto 96 well ELISA plates. Non-specific binding was blocked, and the plates were incubated with primary antibody overnight at 4 °C. Each plate also contained control wells in which tissue culture conditioned media was substituted for the primary antibody. The plates were further incubated with peroxidase-conjugated secondary antibody (1:1000, Jackson Labs) for 1 h and then with 2,2′-azino-di-3-ethylbenzthiazoline (ABTS, KPL) substrate for 30 min. The optical density of each well was determined at 405 nm. Optical density was plotted against protein concentration (Softmax, Molecular Devices) and the linear portion of the curve determined for each sample. The mean linear range for the antibodies used was 64-fold. To compare immunoreactivity between samples the amount of protein required to give an optical density reading of 0.5 was determined. Samples were run twice on different days, with mean values used for analysis. Between-run correlations ranged from 0.91–0.98 and within-run coefficients of variation ranged from 5–7%.

### 2.4. Immunoblotting

Immunoblotting studies were performed to confirm the specificity of the antibodies used. Brain homogenates were separated on 10% SDS polyacrylamide gels. In addition, lysates from PC12 and HTB-17 cells (American Type Culture Collection), neuronal and astrocyte cell lines respectively, were included on the same gels to provide positive and/or negative controls. Following transfer to PVDF, blots were incubated with monoclonal antibodies against MBP (SMI-94, 1:2500), PNF (SMI-34, 1:2500), SNAP-25 (SP12, 1:10) or GFAP (SMI-22, 1:5000). Bands were detected using ECL (GE Healthcare) and imaged using a Fuji LAS-3000 imager (FujiFilm).

### 2.5. $^1$H NMR spectroscopy

Homogenates from the ventral region were centrifuged at 16,000 ×g for 5 min and the pellets discarded. The supernatant was diluted to 500 μl with phosphate buffered saline and $D_2O$ (to 10%), sonicated, and a constant volume of 2 mM 3-trimethylsilyl propionic acid-D4, sodium salt (TSP) standard was added. Solution state NMR spectroscopy was performed using a 9.4 T Bruker Avance 400inv spectrometer with 5 mm BBI-Z inverse broadband probe with Z-gradient coil. Spectra were acquired at room temperature (spectral width 4990 = Hz, time domain = 32 K points, dummy scans = 2, real scans = 1300) using a standard water pre-saturation pulse sequence, followed by a 15 microsecond 90-degree pulse on $^1$H. Our goal was to quantify specific metabolites based on previous studies (Omori et al., 1997; Prabakaran et al., 2004). We were able to identify 11 metabolites in each case based on comparisons with previously published spectra (Govindaraju et al., 2000; Omori et al., 1997) and confirmed using individual metabolite standards. Peak areas were analyzed using the software Dmfit (Massiot et al., 2002). Concentrations of individual metabolites were determined by comparison against the TSP standard peak and calculated as mmol per kg wet weight of tissue used.

### 2.6. Statistical analyses

Normal distributions were confirmed by Shapiro–Wilks tests and logarithmic or square root transformations applied where necessary. Equal variances between groups were confirmed using Levene's test. Correlations between protein or metabolite measures and age, post-mortem interval (PMI), pH and duration of storage were examined using Spearman's Rank analysis. Where significant, confounders were included in the analysis as covariates. Effect size calculations were made using Cohen's *d* statistic (Cohen, 1988) and effect sizes were categorized as small (0.2–0.4), medium (0.5–0.7) or large (0.8 or greater). Statistical analyses were carried out using JMP 5.1.2 (SAS Institute Inc, Cary, NC).

For the protein data, univariate ANOVA was used to compare mean values between groups in the two regions separately. For the metabolites, we carried out two types of analysis. First, as all metabolites were measured in a single assay, we included all measures in a global multivariate ANOVA. This was followed up with individual post-hoc comparisons and calculation of the effect size differences between groups. Secondly, a chemometric analysis was applied to the metabolic measures, using PLS Toolbox for Matlab (Eigenvector Research Inc). We used partial least squares discriminant analysis (PLS-DA), a type of supervised regression analysis. The PLS-DA maximizes the explained variance in the *X* data set (the assays of 11 metabolites), as well as the covariance with the *Y* data set (group identity), creating a model of latent variables describing the maximum separation between the diagnostic groups. We next determined the importance of each of the measures in creating the model by calculating the variable importance in projection (VIP) scores. The model was then used to predict diagnosis of each case based on a 99% confidence limit for class membership. Cross-validation of the model was performed using the "Venetian blinds" method.

## 3. Results

### 3.1. Demographic data

Patient and control groups did not differ in mean age, pH or gender distribution. The mean PMI was shorter in

schizophrenia samples ($t=3.88$, $p<0.001$), and the mean duration of storage was longer ($t=4.21$, $p<0.001$). In the ventral region PNF levels correlated with age (rho = 0.467, $p=0.012$) and PMI (rho = 0.438, $p=0.020$). No other protein measures showed significant correlations with age, PMI, pH or duration of storage. Lactate levels were inversely correlated with pH (rho = −0.576, $p=0.001$) as expected from previous studies (Halim et al., 2008). Lactate is a major determinant of brain pH; therefore we did not use pH as a covariate in the metabolite analysis. No other metabolite measures showed significant correlations with age, PMI, pH or duration of storage. Drug histories were available for 13 of the 15 cases. All had been treated in the past with both low and high potency antipsychotics. All brain samples were tested for the presence of drugs and antipsychotic drugs were detected in only two samples.

### 3.2. Protein levels

No significant differences were observed between the schizophrenia and control groups in any myelin, astrocyte or axonal marker protein in either the dorsal or ventral ALIC (Table 2). Addition of age and PMI as covariates in the analyses of PNF in the ventral region did not affect the results.

### 3.3. Immunoblotting

For each antibody immunoreactive bands were observed at the expected molecular weights (Fig. 1). In human brain homogenates an 18 kDa band was immunoreactive for MBP, representing the major isoform present in the adult human brain. A lower molecular weight band was also present, which represents a minor isoform. As expected, no immunoreactivity for MBP was detected in PC12 or HTB-17 cells. SMI34 (anti-PNF) recognises the phosphorylated forms of neurofilament heavy, and to a lesser extent neurofilament medium. In the postmortem samples immunoreactive bands were present at

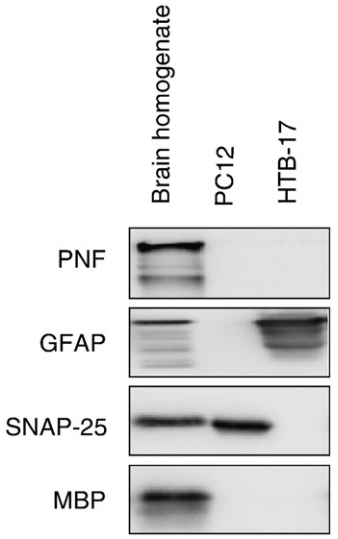


**Fig. 1.** Immunoblotting studies indicate bands at the expected molecular weights for PNF (SMI-34), GFAP (SMI-22), SNAP-25 (SP12) and MBP (SMI94) in representative samples of human brain homogenate, PC12 and HTB-17 cell lysates.

**Table 2**
Protein and metabolic measures in internal capsule in schizophrenia and control samples.

| | Control | Schizophrenia | *F* value | *p* value | Effect size |
|---|---|---|---|---|---|
| | Mean +/− SD (*n*) | Mean +/− SD (*n*) | | | |
| *Myelin markers* | | | | | |
| MOP dorsal | 0.085 +/− 0.033 (10) | 0.105 +/− 0.030 (15) | 2.500 | 0.128 | −0.63 |
| MOP-ventral | 0.103 +/− 0.024 (10) | 0.103 +/− 0.026 (15) | 0.001 | 0.975 | 0.01 |
| *Astrocyte marker* | | | | | |
| GFAP-dorsal | 0.282 +/− 0.081 (13) | 0.294 +/− 0.036 (15) | 0.016 | 0.900 | −0.19 |
| GFAP ventral | 0.288 +/− 0.089 (13) | 0.307 +/− 0.057 (15) | 0.497 | 0.487 | −0.25 |
| *Axonal markers* | | | | | |
| SNAP-25-dorsal | 0.974 +/− 0.257 (13) | 0.923 +/− 0.307 (15) | 0.225 | 0.639 | 0.18 |
| SNAP-25-ventral | 1.188 +/− 0.303(13) | 1.113 +/− 0.260 (15) | 0.509 | 0.482 | 0.27 |
| PNF-dorsal | 1.359 +/− 0.174(13) | 1.357 +/− 0.380 (15) | 0.347 | 0.561 | 0.01 |
| PNF-ventral | 1.462 +/− 0.106(13) | 1.438 +/− 0.118(15) | 0.338 | 0.566 | 0.21 |
| *Metabolic markers* | | | | | |
| Myo-inositol ($^2$CH, 4.05 ppm) | 10.11 +/− 2.26 (13) | 8.66 +/− 3.67 (15) | 1.534 | 0.227 | 0.48 |
| Glycerophosphorylcholine (choline moiety, N($CH_3$)$_3$, 3.21 ppm) | 1.19 +/− 043 (13) | 1.18 +/− 0.47 (15) | 0.021 | 0.887 | 0.02 |
| Phosphorylcholine (N($CH_3$), 3.21 ppm) | 144 +/− 0.26 (13) | 1.39 +/− 0.50 (15) | 0.130 | 0.721 | 0.13 |
| Choline (N($CH_3$)$_3$, 3.19 ppm) | 0.87 +/− 0.30 (13) | 0.67 +/− 0.36 (15) | 2.508 | 0.125 | 0.60 |
| Creatine/phosphocreatine (N($CH_3$), 3.03 ppm) | 8.04 +/− 1.80 (13) | 7.34 +/− 2.10 (15) | 0.882 | 0.356 | 0.36 |
| Succinate ($^2CH_2$, 2.40 ppm) | 0.90 +/− 0.34 (13) | 0.85 +/− 0.29 (15) | 0.080 | 0.779 | 0.16 |
| N-acetylaspartylglutamate (acetyl moiety, $^2CH_3$, 2.04 ppm) | 3.98 +/− 1.33 (13) | 3.90 +/− 1.42 (15) | 0.045 | 0.834 | 0.06 |
| N-acetylaspartate (acetyl moiety, $^2CH_3$, 2.01 ppm) | 3.94 +/− 1.08 (13) | 3.40 +/− 1.06 (15) | 1.808 | 0.190 | 0.50 |
| Acetate ($^2CH_3$, 1.90 ppm) | 4.32 +/− 1.27 (13) | 3.76 +/− 1.51(15) | 1.128 | 0.298 | 0.40 |
| Alanine $^3CH_3$, 147 ppm) | 3.50 +/− 1.09 (13) | 2.67 +/− 0.81(15) | 4.995 | 0.034 | 0.86 |
| Lactate ($^3CH_3$, 1.31 ppm) | 22.14 +/− 1.45 (13) | 18.02 +/− 4.76 (15) | 4.325 | 0.048 | 1.17 |

The MBP assay did not provide reliable data in 3 control samples; these were eliminated from the analysis. Myelin, astrocyte and axonal markers represent the amount of sample required to produce a standard optical density reading; higher values represent lower antigen concentrations. Metabolic marker concentration is expressed as mmol per kg wet weight of tissue used. Effect sizes have positive values if the marker was present at a lower level in schizophrenia samples than in control samples.

200 kDa and 150 kDa respectively. Again, as expected, PC12 and HTB-17 cells were negative for PNF (Giasson and Mushynski, 1996). A single band at 25 kDa was immunoreactive for SNAP-25, being present in the brain homogenates and in the PC12 neuronal cell line, but not in the HTB-17 astrocyte cells. Multiple GFAP bands were observed in the brain homogenates and in the HTB-17 astrocyte cell line, but not in the neuronal cell line. A major band was present at approximately 50 kDa, with a number of fainter bands appearing at lower molecular weights, which may represent alternative isoforms, post-translational modifications or proteolytic fragments, as previously reported (Nicholas et al., 2004).

### 3.4. Metabolite levels

A representative $^{1}$H NMR trace from a control case is shown in Fig. 2A. Summaries of metabolite concentrations are listed in Table 2. The multivariate ANOVA indicated a statistically significant difference between groups in the overall composition of metabolites ($F=2.685$, $p=0.036$). Post-hoc tests revealed significantly lower lactate and alanine

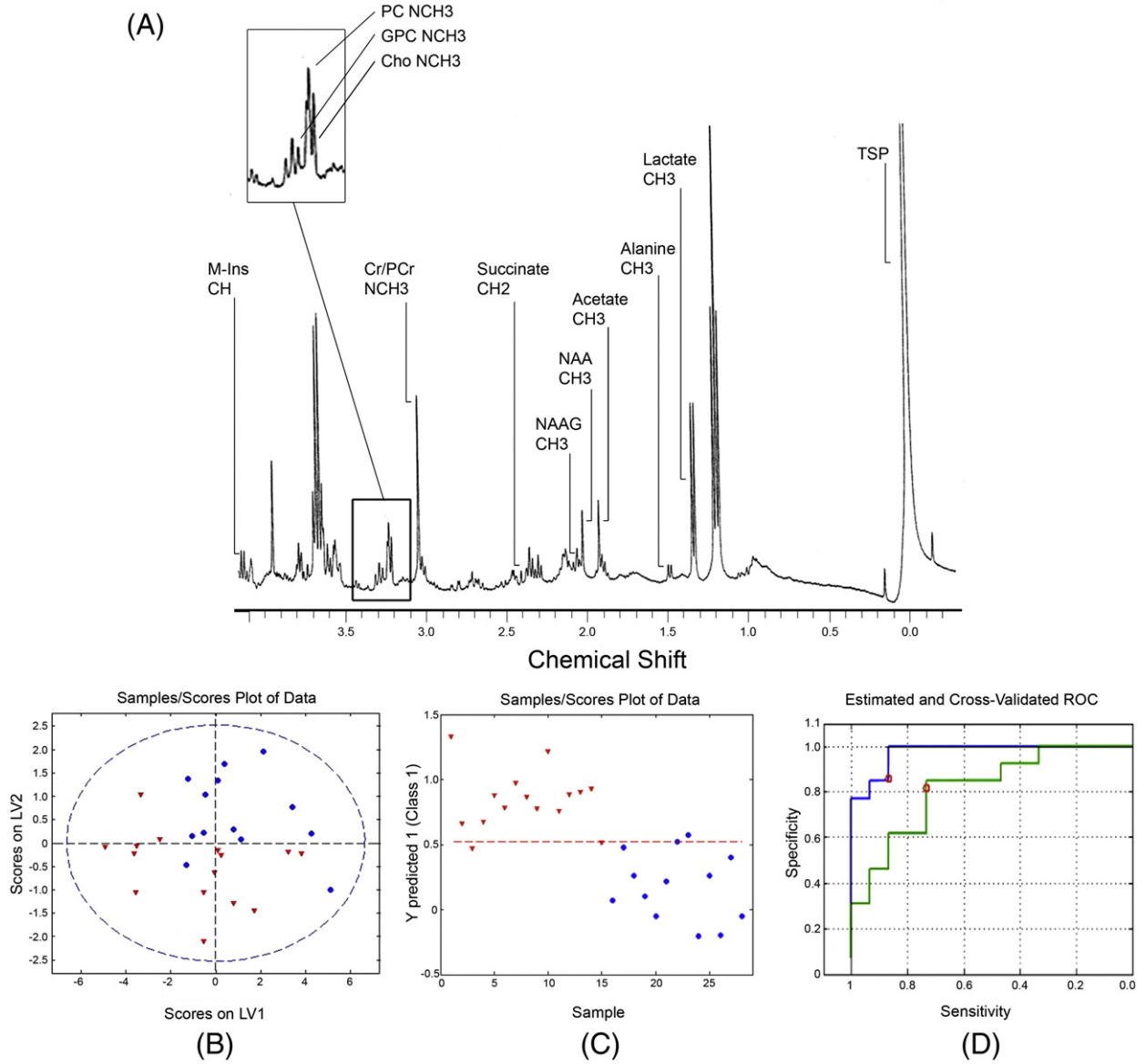


**Fig. 2.** Metabolites in ventral internal capsule. (A) $^{1}$H NMR spectrum of ventral internal capsule. M-Ins: myo-inositol, GPC: glycerophosphorylcholine, PC: phosphorylcholine, Cho: choline, Cr/PCr: creatine/phosphocreatine, NAAG: N-acetylaspartylglutamate, NAA: N-acetylaspartate, TSP: 3-trimethylsilyl propionic acid. (B) PLS-DA model showing separation of samples using scores on latent variables LV1 and LV2. The dashed blue circle represents the 99% confidence interval. (C) Scores for prediction of class (*Y* predicted score) for each sample. In plots B and C control samples are represented as blue circles and schizophrenia samples as red triangles. (D) Receiver operating characteristic (ROC) plots for the original (blue) and the cross-validated (green) PLS-DA models. The red circles represent the values for specificity and sensitivity based on optimum Bayesian-determined threshold derived by the software (red dashed line on plot C). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

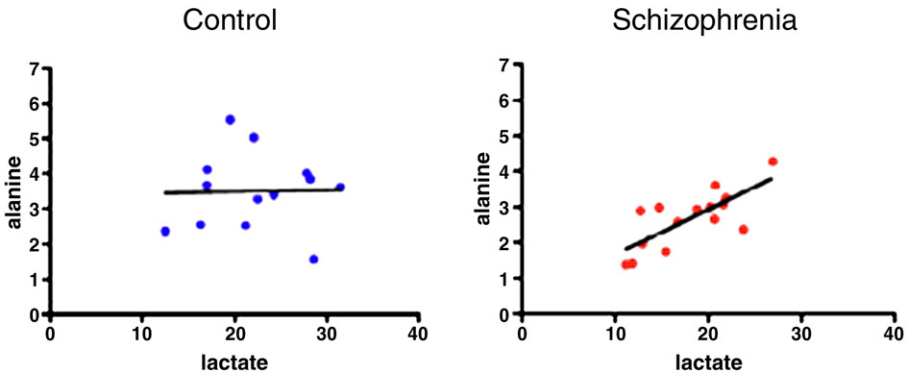


**Fig. 3.** Plots of the relationship between alanine and lactate in the ventral internal capsule in control (blue) and schizophrenia (red) samples. A statistically significant relationship was seen only in the schizophrenia samples. Concentrations are mmol per kg wet weight of tissue. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

concentrations in the schizophrenia group. Both of these metabolites showed large effect sizes. Levels of lactate and alanine were highly correlated in the schizophrenia samples (rho = 0.707, $p = 0.003$), but not in the control group (rho = 0.022, $p = 0.943$) (Fig. 3).

PLS-DA was used to determine if the metabolite data could be used to predict group membership. The scores plot of the first and second latent variables of the model, which accounted for 68.3% of the data variation, demonstrated that some separation could be achieved between the two groups (Fig. 2B). The *Y* predicted score (class membership) for each sample in the initial model is shown in Fig. 2C. The relative influence of each metabolite on the model was examined by comparison of the VIP values. NAAG, choline, phosphocholine, lactate and alanine were found to be important predictors, all of which had VIP values greater than 1.0. The variables with the largest contribution were lactate and alanine, which also had the largest effect sizes for differences between groups. The initial model had a sensitivity of 0.87 and a specificity of 0.92 and the cross-validated model had a sensitivity of 0.73 and a specificity of 0.81 (Fig. 2D).

## 4. Discussion

The central finding of the present study is that metabolite levels in fronto-striatal-thalamic white matter pathways differ in schizophrenia compared with control subjects. This was due to lower levels of lactate and alanine in the schizophrenia group. Our finding of lower lactate is consistent with an investigation of metabolite levels in cerebrospinal fluid of first-episode patients, which found low lactate and elevated glucose (Holmes et al., 2006). In these patients the metabolic disturbances were ameliorated following treatment. While two earlier NMR studies of frontal cortex and thalamus reported no statistically significant differences in these metabolites in schizophrenia (Omori et al., 1997; Prabakaran et al., 2004), small reductions in both lactate and alanine levels were noted in the thalamus (Omori et al., 1997). However, our finding of lower lactate in ventral ALIC differs from two previous post-mortem studies that found higher lactate levels in frontal white matter (Prabakaran et al., 2004) and cerebellum (Halim et al., 2008) in schizophrenia. Glycogen is rapidly converted into lactate post-mortem. Halim and colleagues propose that increased lactate could reflect an elevation in glycogen levels due to antipsychotic treatment. It should be noted that in the previous two studies (Halim et al., 2008; Prabakaran et al., 2004) the schizophrenia group had a lower mean pH than the controls and were obtained from patients with high lifetime prescribed doses of antipsychotic medication. In contrast, in the present study pH was slightly higher in the schizophrenia group and only two of our samples had detectable levels of antipsychotic drugs.

In addition to low levels of lactate, we also observed decreased alanine levels. Low levels of both lactate and alanine in white matter could indicate an alteration in astrocyte–neuron metabolic coupling. During periods of high neuronal activity lactate is released from astrocytes and is taken up by axons as an energy source for sustaining axon excitability, while alanine moves from neurons to astrocytes as part of the transfer of ammonia generated through the synthesis of glutamate (Tekkok et al., 2005; Waagepetersen et al., 2000). In white matter, glycolytic activity (which produces lactate) exceeds oxidative tricarboxylic acid cycle activity (Morland et al., 2007). Of note, in schizophrenia white matter uptake of flurodeoxyglucose is higher than in control subjects, in contrast to grey matter where uptake is lower in patients (Buchsbaum et al., 2007). We propose that high metabolic activity in white matter in schizophrenia could result in lactate depletion. Low levels of both lactate and alanine were previously seen in a rat model of absence seizures when the animals were maintained on a ketogenic diet (Melo et al., 2006). These rats also had elevated brain glucose and astrocytic metabolism was increased.

Finally, we used the metabolite data to produce a model to assess whether the post-mortem pattern of metabolites could be used to distinguish patients from controls. This strategy has previously been used to discriminate schizophrenia and control groups (Holmes et al., 2006; Prabakaran et al., 2004). Our model used available metabolite data from all samples and, as expected, good sensitivity and specificity were obtained for the samples used to construct the model. A statistical approach to validating the model also yielded a reasonably robust result, but a new series of samples is required to test the model rigorously.

We were unable to detect group differences in proteins representing myelin, axons or astrocytes in either ventral or dorsal regions of the ALIC. To date there have been few studies

of myelin-associated proteins, or the mRNAs that encode them, in white matter in schizophrenia, and findings are inconsistent. In a previous study we found lower MBP protein levels in frontal grey matter in schizophrenic patients who died by suicide (Honer et al., 1999), although we observed no deficit in visual association cortex in a second study (Beasley et al., 2005), while MBP mRNA levels were also not changed in cingulate grey matter (Dracheva et al., 2006). MBP protein levels have not been quantified in white matter in schizophrenia, although altered levels of other myelin-associated proteins/mRNAs have been reported. Lower levels of 2′, 3′-cyclic nucleotide 3′-phosphodiesterase (CNP) were noted in a proteomics study of prefrontal white matter (Prabakaran et al., 2004). Furthermore, lower expression of myelin-associated glycoprotein (MAG) and CNP transcripts in anterior cingulate white matter was reported in schizophrenia (McCullumsmith et al., 2007), although no difference in MAG or CNP mRNA expression was found in prefrontal white matter (Mitkus et al., 2008). We also report no difference in levels of the astrocyte marker GFAP between groups, consistent with a previous immunocytochemical study in frontal white matter (Falkai et al., 1999), and suggestive of a lack of ongoing neurodegeneration in this brain region. No group differences in levels of PNF or SNAP-25 were observed in the present study. Serine residues are heavily phosphorylated on axonal neurofilaments, compared with dendritic and perikaryal neurofilaments, and therefore PNF antibodies have been identified as axonal markers. SNAP-25 is a presynaptic protein that is transported in axons, and is lower in cortical grey matter in schizophrenia (Honer et al., 2002). Levels of SNAP-25 in white matter are increased in Alzheimer's disease, suggesting an impairment of axonal transport (Dessi et al., 1997).

In summary, by focussing on the ALIC, we were able to detect differences in the white matter component of the fronto-striatal-thalamic circuitry in schizophrenia. Relationships between the metabolic function of neurons and glial cells may be a fruitful source of investigation to understand the mechanism of illness in schizophrenia.

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**Contributors**

Drs. Beasley and Honer designed the study and provided intellectual contributions. Drs. Dwork, Rosoklija, Mann, Mancevski, Jakovski and Davceva provided the tissue for the study. Dr. Beasley performed the ELISA studies. Mr. Tait and Dr. Straus performed the NMR study. Dr. Beasley performed the statistical analyses and wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

**Conflict of interest**

All authors declare they have no conflicts of interest.

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