Region-specific changes in phospholipid metabolism in chronic, medicated schizophrenia

31P-MRS study at 4.0 Tesla

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Background Membrane phospholipid abnormalities in people with schizophrenia, measured with 31P magnetic resonance spectroscopy (31P-MRS), have been previously reported in brain regions involved in this disorder.

Aims In this 4.0 Tesla 31P-MRS study of people with schizophrenia, membrane phospholipid metabolism was examined in brain regions previously inaccessible due to their small volumes.

Method Three-dimensional chemical-shift imaging (3D–CSI) examined 15 cc volumes in 12 brain regions in 11 people with chronic schizophrenia and 11 healthy control volunteers.

Results Glycerophosphoethanolamine was decreased in the anterior cingulate, right prefrontal cortex and left thalamus, but increased in the left hippocampus and cerebellum in those with schizophrenia. Phosphoethanolamine and glycerophosphocholine were decreased in the right prefrontal region and phosphocholine was decreased in the anterior cingulate. No significant difference in membrane phospholipid levels existed between groups in the parieto-occipital and posterior cingulate regions.

Conclusions Altered membrane phospholipid metabolism was demonstrated in all regions implicated in schizophrenia.

Declaration of interest This work was funded by the Canadian Institute of Health Research Grant MT–1078.

Brain structures implicated in schizophrenia include the prefrontal cortex (Selemon & Goldman-Rakic, 1999), the anterior cingulate (Benes, 1998), the thalamus (Jones, 1997), the hippocampus (Lipska & Weinburger, 1993), the cerebellum (Andreasen, 1999) and the neuronal circuits connecting these structures (Carlsson & Carlsson, 1990; Friston, 1998; Grace et al, 1998; Feinberg & Guazzelli, 1999). Phosphorus magnetic resonance spectroscopy (31P-MRS) measures membrane (phosphomonoesters (PME); phosphodiesters (PDE)) and energy (adenosine triphosphate (ATP); phosphocreatine (PCr)) metabolism. Past 31P-MRS of patients with schizophrenia who had never been treated shows decreased PME and increased PDE in the left prefrontal and left temporal regions. Studies of people with chronic schizophrenia are inconsistent with unchanged or decreased PME in prefrontal and temporal locations and increased, unchanged or decreased PDE in prefrontal, parietal and temporal locations (Williamson & Drost, 1999). Past 31P-MRS at low fields had limited spatial resolution. This high-field study images effective voxels of 15 cc and measures each PME metabolite – phosphoethanolamine (Peth) and phosphocholine (PCh) and PDE metabolite – glycerophosphoethanolamine (GPEth) and glycerophosphocholine (GPCh) from multiple locations. We hypothesised that people with schizophrenia would show altered membrane phospholipid metabolism in structures implicated in schizophrenia – the dorsolateral–prefrontal cortex, the anterior cingulate, the thalamus, the hippocampus and the cerebellum.

METHOD

Subjects

Eleven male patients with chronic schizophrenia and 11 healthy male volunteers participated in the study after giving informed consent according to the guidelines of the Review Board for Health Sciences Research Involving Human Subjects at the University of Western Ontario (see Table 1). The DSM–IV diagnosis of schizophrenia was established with the Structured Clinical Interview for DSM–IV (SCID) (First et al, 1997) administered by a psychiatrist (P.C.W., Y.S). Four were classified as having paranoid, five residual and two undifferentiated schizophrenia. Six patients were on olanzapine (10.8 mg, s.d.=5.3), two were taking conventional neuroleptics (fluphenazine decanoate 25 mg intramuscular every three weeks, flupenthixol decanoate 20 mg intramuscular every four weeks), one was taking clozapine 350 mg four times daily, one was taking quetiapine 450 mg four times daily and one ziprasidone 120 mg four times daily at the time of the scan and all had been on conventional medications prior to going on atypical neuroleptics. Four had received an anticholinergic agent and one benzodiazepine in the 24 hours before the scan. Ratings of symptoms were assessed with the Scale for the Assessment of Negative Symptoms (SANS) (Andreasen, 1984a) and the Scale for the Assessment of Positive Symptoms (SAPS) (Andreasen, 1984b) without knowledge of 31P-MRS results. Parental education of the most educated parent was rated on a four-point scale as in our previous publications (Stanley et al, 1995; Potwarka et al, 1999a). Handedness was also assessed by a rating scale (Bryden, 1977).

The healthy volunteers were recruited from the community by advertisement and were assessed by a psychiatrist (P.C.W., Y.S.) with the SCID. Controls were of comparable age, gender, handedness and parental education levels. No patients or controls had a history of head injury, drug or alcohol misuse or serious medical illnesses.

1H-MRI

All experiments were performed on a 4.0 Tesla (Varian/Siemens/Unity-INOVA) whole-body magnetic resonance scanner operating at 170.3 MHz. A single-tuned, 1H quadrature hybrid-birdcage volume head-coil was used for shimming and imaging. Subjects were positioned supine with their heads secured in a plexiglas cradle and a $10 \times 10 \times 6$ cm (6 cm superior–inferior) volume positioned mid-sagittally was shimmed with an automated shim protocol (Gruetter,

Table I Subject characteristics

	Control group	Schizophrenia group
Male	II	II
Female	0	0
Right-handed	9	9
Age (years)	42.2 (s.d.=8.5)	42.9 (s.d.=7.4)
Parental education[1]	2.4 (s.d.=1.2)	1.7 (s.d.=7.3)
Length of illness (years)		21.6 (s.d.=7.3)
Total SANS score		28.9 (s.d.=11.7)
Total SAPS score		16.4 (s.d.=6.0)

1. Education level was rated as follows: 1, ≤Grade 10 (age 16); 2, Grades 11–13 (ages 17–19); 3, 1–3 years in college; and 4, ≥4 years in college.

1993). A 2D-FLASH imaging sequence acquired anatomical sagittal and coronal images of the brain for ^{31}P voxel positioning (TR=11 ms, TE=6 ms, 256 phase-encodes, 0.93×0.93 mm pixels). Thirty-two T_1-weighted transverse images were acquired on the brain using a magnetisation-prepared (adiabatic inversion pulse 0.5 secs prior to data collection) 3D-FLASH imaging sequence (TR=11 ms, TE=6.2 ms, total acquisition time <3 minutes) with a nominal slice thickness of 4.375 mm and 1.09×1.09 mm in-plane resolution. These images were used to calculate grey matter, white matter and cerebrospinal fluid (CSF) ratios in the ^{31}P-CSI volumes.

Image segmentation/partial-volume estimation

The transverse image data set for each subject was first separated into three distinct binary tissue maps: grey matter, white matter and CSF/air/bone by thresholding the original high-contrast image set, which best displayed the ventricles, corpus callosum and grey matter of the anterior cingulate. The thresholding co-efficients were then varied until each segmented image type matched the corresponding anatomy in the original reference image. The thresholding values differed between subjects since image signal intensity depended on receiver gain and coil loading. To obtain the tissue ratio contribution for each ^{31}P region of interest (ROI), the three segmented image data sets were convolved with the calculated 3D-PSF of the ^{31}P-CSI acquisition (Jensen *et al*, 2001). The partial volume contribution for each tissue type was then expressed as a percentage of total tissue contribution for each ROI.

In vivo ^{31}P-MRS

Once the ^{1}H-shimming and imaging were completed, the ^{1}H-head coil was manually replaced with a single-tuned, ^{31}P quadrature, 14 cm long birdcage head-coil with the subject still in place in the plexiglas cradle inside the magnet. Previous phantom experiments verified that manual coil replacement did not affect the shim. A ^{31}P external reference standard (methylene diphosphonic acid (MDP), 270 mM, T1@ 4.0T∼6 secs) was fastened to the plexiglas head cradle just left of the patient's head The 1.5 cm diameter reference tube was positioned so its length ran axially, spanning the sensitive region of the ^{31}P-RF coil. Transmit/receive frequency was centred on PCr, as measured with a global free induction decay (FID). Tip angle, optimised for the PME/PDE resonance at a 500 ms TR, was 32 degrees. *In vivo* ^{31}P-MRS utilised an optimised 3D-CSI sequence: TR=500 ms; Rx bandwidth=±2 kHz; complex-points=1600; readout duration=400 ms; pre-pulses=20; Rx gain=94 dB; pre-acquisition delay=1.905 ms; field of view (FOV) (*x*,*y*,*z*)=280 mm; nominal volume=5.4 cc; maximum phase-encode matrix dimension (*x*,*y*,*z*) $14 \times 14 \times 14$ (zero-filled out to $16 \times 16 \times 16$ prior to reconstruction). The 3D-CSI sequence used a reduced phase-encoding scheme based on prior work (Ponder *et al*, 1994). This scheme allows for the inclusion of spherically bound, reduced-point, weighted *k*-space acquisition, providing approximately 40% more signal-to-noise for a given scan time and effective voxel volume over conventional methods.

In vivo post-processing and spectral analysis

All *in vivo* CSI/image data were processed and viewed using Varian Nuclear Magnetic Resonance (VNMR) software, Version 6.1b and software designed and written on site. Prior to Fast Fourier Transform (FFT) reconstruction to spatially resolve the CSI spectra, the collected *k*-space data was centred in a $16 \times 16 \times 16$ cubic matrix. Each time-domain FID was then zero-filled out to 2048 complex points and left-shifted five points to remove residual bone/rigid membrane signal.

Using the ^{1}H images, the 3D-CSI data grid was shifted in the *x*, *y* and *z* dimensions in order to position each voxel in the appropriate ^{31}P ROI. For all subjects, the Brodmann area and Talairach co-ordinates for voxel centres (*x*,*y*,*z*) in the brain were evaluated for every ^{31}P ROI using SPM99b-Statistical Parametric Mapping (Ashburner *et al*, 2000) and are listed in Table 2. Partial overlap with adjacent structures was unavoidable in many of the regions due to voxel volume and shape (Fig. 1).

All *in vivo* spectra were fit in the time-domain using a non-linear, iterative fitting program developed on site. The fitting routine is based on the Marquardt–Levenberg algorithm, utilising prior spectral knowledge for the relative amplitudes, linewidths, lineshapes, peak positions and J-coupling constants to model the *in vivo* ^{31}P brain spectrum (Potwarka *et al*, 1999b). Since it was positioned along the inner face of the coil, the reference standard was subject to different RF power than the subject and was volume-bound by the *x* and *y* tube dimension (1.5 cm diameter), both requiring correction. Therefore, the MDP reference area, fitted with a single Gaussian peak, was corrected for T_1-saturation, RF coil sensitivity and volume. Each ^{31}P metabolite area was also T_1-corrected, then normalised to the MDP signal and finally corrected by the brain water fraction of 75% to obtain absolute millimolar values per unit brain water (mM/L H_2O) for each ^{31}P metabolite. Human *in vivo* ^{31}P metabolite T_1-values at 4.1T for Pi, PCr and ATP of 1.59 secs, 2.39 secs and 0.79 secs were used for the T_1-correction (Heatherington *et al*, 2001). T_1-values averaged from the literature at 1.9–2.0T of 2.35 secs and 2.14 secs for the PME and PDE metabolites, respectively, were also used (Boska *et al*, 1990; Bottomley *et al*, 1994; Oberhaensli *et al*, 1987). These values agreed closely with our own *in vivo* T_1-values at 4.0 Tesla obtained from a global FID with a 90 degree pulse and TR values ranging from 0.5 to 20 secs. The fitted metabolite

amplitudes are not T_2-weighted since the fitting algorithm back-extrapolates to time zero. No partial volume corrections were made to any of the derived ^{31}P metabolite concentrations.

Statistics

All statistical calculations used SPSS version 10.0 for Windows. A two-tailed *t*-test was used to test for significant differences in age, parental education, grey matter, white matter and CSF partial volumes between groups ($P < 0.05$). Statistical treatment of all 10 ^{31}P metabolite ratios for each region first involved a one-factor MANCOVA including the covariate of age with a between-subjects factor of group. ANCOVA was used to detect what individual metabolites differed significantly ($P < 0.05$) between groups. In each region where significant group differences in ^{31}P were found, Pearson correlations were computed, within the patient group, between ^{31}P metabolite levels and each of the following variables: SANS and SAPS scores, age, parental education, duration of illness and ^{31}P metabolite levels in all regions where significant group differences in ^{31}P metabolites were found. Significance for the Pearson correlate was set to $P < 0.01$.

RESULTS

No significant differences were found in age, parental education level or handedness between people with medicated chronic schizophrenia and healthy controls (see Table 1). Grey matter partial volume was significantly reduced by 15% in the left hippocampus of the schizophrenia group ($P = 0.04$), compared with the control group. Partial volumes of grey matter, white matter and CSF did not differ significantly in any of the other regions studied. MANCOVA showed a significant difference between groups in the left thalamus (Wilk's lambda=0.249, $F = 3.016$, d.f.=10,10, $P = 0.048$) and a trend was seen in the left hippocampus (Wilk's lambda=0.278, $F = 2.602$, d.f.=10,10, $P = 0.074$). The ANCOVA revealed significant alterations of PME and PDE metabolites in several regions in the schizophrenia group (see Table 3). There were no significant correlations between age, duration of illness, parental education, SANS or SAPS scores and any of the significant phosphorus findings. However, a

Table 2 Brodmann areas and Talairach coordinates for regions of interest

Region	Brodmann	Talairach		
		x	*y*	*z*
Anterior cingulate	32	0	44	2
Posterior cingulate	23	0	−48	26
Left pre-frontal	10	−22	42	2
Right pre-frontal	10	22	42	2
Left thalamus		−9	−11	5
Right thalamus		9	−11	5
Left parieto-occipital	39	−31	−56	26
Right parieto-occipital	39	31	−56	26
Left cerebellum		−20	−31	−26
Right cerebellum		20	−61	−26
Left hippocampus		−24	−29	−12
Right hippocampus		24	−29	−12

negative correlation was found in the anterior cingulate cortex between membrane phospholipid (Potwarka *et al*, 1999*a*) and duration of illness ($r = −0.761$, d.f.=9, $P = 0.007$) and GPCh correlated negatively with SANS in the right parieto-occipital cortex ($r = −0.774$, d.f.=9, $P = 0.005$).

DISCUSSION

The most striking finding of this study is that people with chronic schizophrenia showed membrane phospholipid differences from controls on both sides of the brain in all regions implicated by current models of
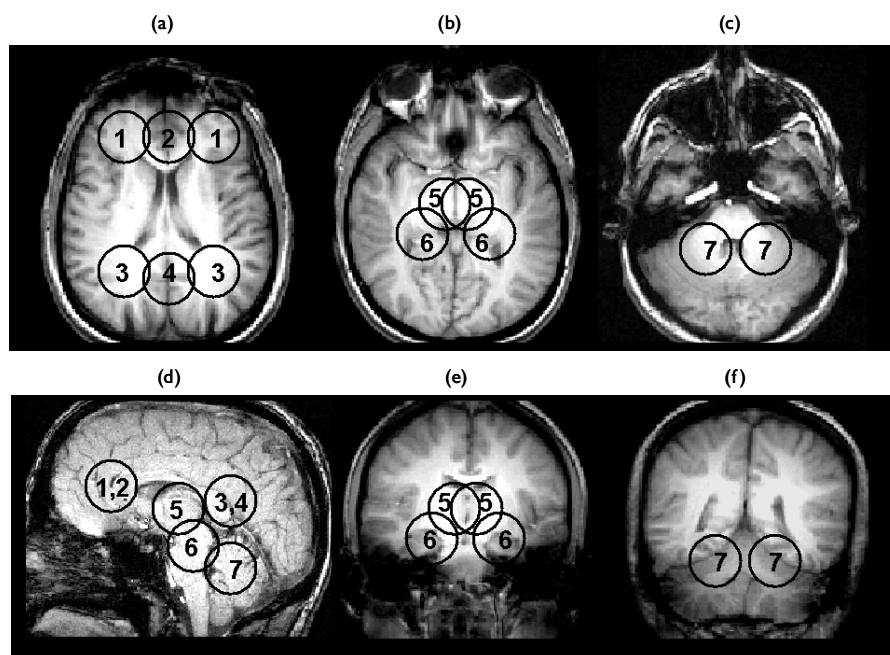
Fig. 1 Voxel positions. Regions of interest displayed on transverse localiser images. Pre-frontal, IA; anterior cingulate, 2A; parieto-occipital, 3A; posterior cingulate, 4A; thalamus, 5B; hippocampus, 6B; cerebellum, 7C. Corresponding voxel positions are displayed on sagittal (D) and coronal images (E, F). Effective voxels shown represent actual size with respect to images and are 3.1 cm in diameter (15 cc volume).

schizophrenia suggesting that, in at least the later stages of illness, there is not a selective involvement of a particular region or neuronal circuit. GPEth appears to be decreased in the anterior cingulate, right prefrontal cortex and left thalamus but is increased in the left hippocampus and left cerebellum compared to controls. PEth and GPCh were decreased in the right prefrontal region and PCh was decreased in the anterior cingulate cortex. No significant differences in membrane phospholipid levels were seen between groups in the parieto-occipital and posterior cingulate regions as expected. Although some differences would be expected due to chance alone, this is unlikely in most of these regions as the MANCOVA indicated significant differences between groups in the left thalamus with a trend in the left hippocampus.

IMPLICATIONS OF ALTERED PHOSPHODIESTER LEVELS

The PDE metabolites: GPCh and GPEth are both products of phospholipase A1 and A2 activity. Processes that can increase PDE levels in the brain involve decreased phosphodiesterase activity and/or increased phospholipase A1 and A2 activity which have been suggested to play a role in the development of schizophrenia (Horrobin *et al*, 1994). However, this would not explain the decrease in GPEth in the anterior cingulate, right prefrontal cortex and left thalamus. We have previously observed increased prefrontal PDE in patients with first-episode schizophrenia but not in patients with chronic schizophrenia, suggesting a degenerative process (Stanley *et al*, 1995). Decreased PDE metabolites in this region in the present study in patients with chronic schizophrenia are consistent with such a process but the finding of increased PDE metabolites in the left hippocampus and left cerebellum could indicate that membrane breakdown continues in these regions long after the onset of the illness. Further studies of patients with first-episode schizophrenia using the present methodology are necessary to support this possibility.

OTHER METABOLITES

Only two significant differences were seen between groups in high-energy phosphates: decreased PCr in the parieto-occipital

region and decreased ATP in the left hippocampus in keeping with earlier studies (Williamson & Drost, 1999). We also did not observe changes in MP which we had found in chronic patients in the medial prefrontal region in a previous study (Potwarka *et al*, 1999a) which might be explained by the much smaller voxel (15 cc *v*. 60 cc) and a higher field strength which decreases this peak due to chemical shift anisotropy (Kilby *et al*, 1991). Few correlations were seen between symptoms and ^{31}P-MRS metabolites as also seen in

previous studies (Williamson & Drost, 1999).

Effects of medication

Patients were chronic and on neuroleptic medication. However, two recent ^{31}P-MRS studies demonstrated that neuroleptic medications decreased PDE levels in the temporal lobe (Fukuzako *et al*, 1999) and increased PDE levels in the frontal lobes (Volz *et al*, 1999). As our findings went in the opposite direction in these locations,

Table 3 Significant ^{31}P metabolite differences: people with chronic schizophrenia *v.* matched control subjects

Region	Metabolite	Schizophrenia group (mM/L)	Control group (mM/L)	F	d.f.	P
Anterior cingulate	PCh	0.35 (s.d.=0.25)	0.88 (s.d.=0.53)	8.222	1,19	0.010
	GPEth	0.48 (s.d.=0.22)	0.80 (s.d.=0.18)	13.843	1,19	0.001
Right pre-frontal	PEth	0.84 (s.d.=0.23)	1.15 (s.d.=0.28)	7.397	1,19	0.014
	GPEth	0.51 (s.d.=0.21)	0.85 (s.d.=0.45)	4.553	1,19	0.046
	GPCh	0.84 (s.d.=0.29)	1.10 (s.d.=0.20)	5.468	1,19	0.030
Left thalamus	GPEth	0.27 (s.d.=0.22)	0.71 (s.d.=0.30)	14.140	1,19	0.001
Right parieto-occipital	PCr	2.55 (s.d.=0.40)	3.19 (s.d.=0.62)	7.371	1,19	0.014
Left cerebellum	GPEth	0.92 (s.d.=0.30)	0.57 (s.d.=0.40)	4.962	1,19	0.038
Left hippocampus	GPEth	0.74 (s.d.=0.26)	0.45 (s.d.=0.27)	6.078	1,19	0.023
	α-ATP	2.19 (s.d.=0.39)	2.61 (s.d.=0.47)	5.050	1,19	0.037

ANCOVA results for significant ^{31}P metabolite differences in patients with chronic schizophrenia *v.* matched controls. All ^{31}P metabolite values stated are expressed in units of millimolar (mM) concentration per litre (L) brain water assuming an average brain water fraction of 75%. PCh, phosphocholine; GPEth, glycerophospoethanolamine; PEth, phosphoethanolamine; GPCh, glycerophosphocholine; PCr, phosphocreatine; α-ATP, alpha adenosine triphosphate.
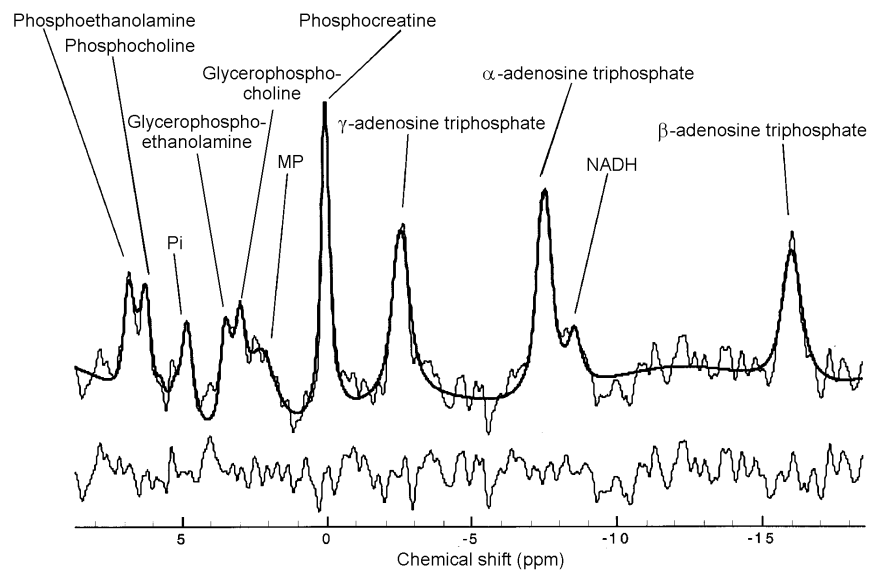
Fig. 2 ^{31}P *in vivo* spectrum from the upper left cerebellum of a healthy control. Raw data are displayed with modelled fit and residual. 15 Hz exponential filtering has been applied for display. Nominal voxel volume is 5.4 cc, effective volume is ~15 cc.

it is difficult to attribute differences to neuroleptics.

Spectroscopy limitations

Due to the short repetition-time (500 ms) in our acquisition, the resulting ^{31}P spectra are T_1-weighted. The observed changes in metabolite levels in schizophrenia may therefore partially result from differing T_1-values in the affected regions, and may not be purely due to concentration differences. However, based on literature T_1 values, T_1 values in people with schizophrenia would have to change considerably, up to 400%, in order to equalise the metabolite ratios between groups. This is unlikely and there is no evidence to date suggesting that brain phosphorus metabolite T_1 values are altered in schizophrenia. Our 15 cc voxels are the smallest used in a schizophrenia ^{31}P-MRS study to date, but this volume is still large resulting in considerable contribution from adjacent tissue in the hippocampus, the anterior cingulate and the thalamus. The fall off in the ^{31}P RF coil B_1-sensitivity prevented ideal positioning of a 15 cc volume in the cerebellum, resulting in partial contribution of the spectra from the occipital lobes (see Fig. 1). However, the nearby voxels entirely within the parieto-occipital region showed no phospholipid changes, rendering it unlikely that our reported change in GPEth in the left cerebellum is from the occipital region.

Partial-volume limitations

People with schizophrenia have been shown to possess less cortical grey matter and more CSF than controls (Zipursky *et al*, 1992). A partial-volume correction was therefore applied to all brain regions showing significant changes in ^{31}P metabolites, using literature estimates for ^{31}P metabolite concentration differences between tissue-type (Buchli *et al*, 1994; Hetherington *et al*, 2001).

Partial volumes of grey matter, white matter and CSF in the anterior cingulate, left thalamus, right parieto-occipital cortex and right prefrontal cortex differed by only 2–5% between the groups, which was not significant. The effect of these small volume differences on the ^{31}P metabolite concentrations is very slight and does not explain the observed group differences in the ^{31}P metabolites in any of these regions. The 15% reduction in grey matter in the left hippocampus of the schizophrenia group, as well

as 13% more white matter in this region, explain only about 4% of the observed 64% increase in GPEth in this group, based on 40% greater PDE levels in white matter (Buchli *et al*, 1994). Although not found to be significant, the 15% increase in grey matter partial volume, as well as the 7% reduction in white matter and 8% reduction in CSF partial volumes, in the left cerebellum in schizophrenia patients cannot account for the observed 60% increase in GPEth in this region.

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REFERENCES

Andreasen, N. (1984a) *Scale for the Assessment of Negative Symptoms.* Iowa City, IA: University of Iowa.

___ **(1984b)** *Scale for the Assessment of Positive Symptoms.* Iowa City, IA: University of Iowa.

___ **(1999)** A unitary model of schizophrenia. *Archives of General Psychiatry,* **56**, 781–793.

Ashburner, J., Fristor, K., Holmes, A., et al (2000) *Statistical Parametric Mapping (SPM99b).* Website: http://www.fil.ion.ucl.ac.uk/spm

Benes, M. F. (1998) Model generation and testing to probe neural circuitry in the cingulate cortex of postmortem schizophrenic brain. *Schizophrenia Bulletin,* **24**, 219–230.

Borka, M. D., Hubesch, D. J., Meyerhoff, D. B., et al (1990) Comparison of ^{31}P-MRS and ^{1}H-MRI and 1.5 and 2.0T. *Magnetic Resonance in Medicine,* **13**, 228–238.

Bottomley, P. A. & Ouwerkerk, R. (1994) Optimum flip angles for exciting NMR with uncertain T^1 values. *Magnetic Resonance in Medicine,* **32**, 137–141.

Bryden, P. (1977) Measuring handedness with questionnaires. *Neuropsychologia,* **15**, 617–624.

Buchli, R., Duc, C. O., Martin, E., et al (1994) Assessment of absolute metabolite concentrations in human tissue by ^{31}P-MRS *in vivo.* Part I: Cerebrum, cerebellum, cerebral gray and white matter. *Magnetic Resonance in Medicine,* **33**, 447–452.

Carlsson, M. & Carlsson, A. (1990) Schizophrenia: a subcortical neurotransmitter imbalance syndrome? *Schizophrenia Bulletin,* **16**, 425–432.

Feinberg, I. & Guazzelli, M. (1999) Schizophrenia — a disorder of the corollary discharge systems that integrate the motor systems of thought with the sensory systems of consciousness. *British Journal of Psychiatry,* **174**, 169–204.

First, M. B., Spitzer, R. L., Gibbon, M., et al (1997) *Structured Clinical Interview (SCID) for DSM–IV Axis I Disorders.* Washington, DC: American Psychiatric Press.

Friston, K. J. (1998) The disconnection hypothesis. *Schizophrenia Research,* **30**, 115–125.

Fukuzako, H., Fukuzako, T., Kodama, S., et al (1999) Haloperidol improves membrane phospholipid

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abnormalities in temporal lobes of schizophrenic patients. *Neuropsychopharmacology*, **21**, 542–549.

Grace, A. A., Moore, H. & O'Donnell, P. (1998) The modulation of corticoaccumbens transmission by limbic afferents and dopamine: a model for the pathophysiology of schizophrenia. *Advances in Pharmacology*, **42**, 721–724.

Gruetter, R. (1993) Automatic, localized *in vivo* adjustment of all first and second-order shim coils. *Magnetic Resonance in Medicine*, **29**, 804–811.

Hetherington, H. P., Spencer, D. D., Vaughan, J. T., et al (2001) Quantitative ^{31}P spectroscopic imaging of human brain at 4 Tesla: Assessment of gray and white matter differences of phosphocreatine and ATP. *Magnetic Resonance in Medicine*, **45**, 46–52.

Horrobin, D. D., Glen, A. I. M. & Vaddadi, K. (1994) The membrane hypothesis of schizophrenia. *Schizophrenia Research*, **13**, 195–207.

Jones, E. G. (1997) Cortical development and thalamic pathology in schizophrenia. *Schizophrenia Bulletin*, **23**, 483–501.

Kilby, P. M., Bolas, N. M. & Radda, G. K. (1991) ^{31}P-NMR Study of brain phospholipid structures *in vivo*. *Biochimica et Biophysica Acta*, **1085**, 257–264.

Lipska, B. K. & Weinberger, D. R. (1993) Delayed effects of neonatal hippocampal damage on haloperidol-induced catalepsy and apomorphine-induced sterotypic behaviors in the rat. *Developmental Brain Research*, **75**, 213–222.

Oberhaensli, R. D., Galloway, G. J., Hilton-Jones, D., et al (1987) The study of human organs by phosphorus-31 topical magnetic resonance spectroscopy. *British Journal of Radiology*, **60** 367–373.

Ponder, S. L. & Twieg, D. B. (1994) A novel sampling method for ^{31}P spectroscopic imaging with improved sensitivity, resolution and sidelobe suppression. *Journal of Magnetic Resonance* (Series B), **104**, 85–88.

Potwarka, J., Drost, D. J., Williamson, P. C., et al (1999a) A ^{1}H decoupled ^{31}P chemical shift imaging study of medicated schizophrenic patients and healthy controls. *Biological Psychiatry*, **45**, 687–693.

__, __ & __ (1999b) Quantifying ^{1}H decoupled *in vivo* ^{31}P brain spectra. *NMR in Biomedicine*, **12**, 8–14.

Selemon, J. D. & Goldman-Rakic, P. S. (1999) The reduced neuropil hypothesis: a circuit based model of schizophrenia. *Biological Psychiatry*, **45**, 17–25.

Stanley, J. A., Williamson, P. C., Drost, D. J., et al (1995) An *in vivo* study of the prefrontal cortex of schizophrenic patients at different stages of illness via phosphorus magnetic resonance spectroscopy. *Archives of General Psychiatry*, **52**, 399–406.

Volz, H.-P., Rössger, G., Riehemann, S., et al (1999) Increase of phosphodiesters during neuroleptic treatment of schizophrenics: a longitudinal ^{31}P-magnetic resonance spectroscopic study. *Biological Psychiatry*, **45**, 1221–1225.

Williamson, P. C. & Drost, D. J. (1999) ^{31}P magnetic resonance spectroscopy in the assessment of brain phospholipid metabolism in schizophrenia. In *Phospholipid Spectrum Disorder in Psychiatry* (eds A. I. M. Glen & D. F. Horrobin), pp. 45–55. Carnforth: Marius Press.

Zipursky, R. B., Lim, K. O., Sullivan E. V., et al (1992) Widespread cerebral grey matter volume deficits in schizophrenia. *Archives of General Psychiatry*, **49**, 195–205.