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Tissue-Specific Differences in Brain Phosphodiesterases in Late-Life Major Depression

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Abstract

Objective—Late-life depression has been hypothesized to have a neurodegenerative component that leads to impaired executive function and increases in subcortical white matter hyperintensities. Phosphorus magnetic resonance spectroscopy (MRS) can quantify several important phosphorus metabolites in the brain, particularly the anabolic precursors and catabolic metabolites of the constituents of cell membranes, which could be altered by neurodegenerative activity.

Methods—Ten patients with late-life major depression who were medication free at time of study and 11 aged normal comparison subjects were studied using ^{31}P MRS three-dimensional chemical shift imaging at 4 Tesla. Phosphatidylcholine and phosphatidylethanolamine comprise 90% of cell membranes in brain but cannot be quantified precisely with ^{31}P MRS. We measured phosphocholine and phosphoethanolamine, which are anabolic precursors, as well as glycerophosphocholine and glycerophosphoethanolamine, which are catabolic metabolites of phosphatidylcholine and phosphatidylethanolamine.

Results—In accordance with our hypotheses, glycerophosphoethanolamine was elevated in white matter of depressed subjects, suggesting enhanced breakdown of cell membranes in these subjects. Glycerophosphocholine did not show any significant difference between comparison and depressed subjects but both showed an enhancement in white matter compared with gray matter. Contrary to our hypotheses, neither phosphocholine nor phosphoethanolamine showed evidence for reduction in late-life depression.

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Conclusion—These findings support the hypothesis that neurodegenerative processes occur in white matter in patients with late-life depression more than in the normal elderly population.

Keywords

MRSI; ^{31}P MRS; elderly; aging; membranes

INTRODUCTION

Biologic membranes serve numerous, essential cellular functions. Phosphatidylcholine and phosphatidylethanolamine are the two most prevalent membrane phospholipids, with each comprising approximately 45% of the central nervous system total membrane phospholipid pool.¹ Phosphorus magnetic resonance spectroscopy (^{31}P MRS) can quantify both anabolic precursors (phosphomonoesters [PMEs]) and catabolic metabolites (phosphodiester [PDEs]) of these two important membrane phospholipids, giving insight into the state of membrane integrity and turnover.^{2,3} The PMEs that can be resolved into independent peaks at 4-Tesla field strength are phosphocholine (PCho) and phosphoethanolamine (PEtn) and the PDEs that can be resolved are glycerophosphocholine (GPCho) and glycerophosphoethanolamine (GPEtn).

Phosphatidylcholine is synthesized directly via the cytidine diphosphate–choline pathway⁴ from PCho. It can also be generated from phosphatidylethanolamine via phosphatidylethanolamine *N*-methyltransferase.^{5,6} In an analogous manner, phosphatidylethanolamine can be synthesized from PEtn via the cytidine diphosphate–ethanolamine pathway (Fig. 1);⁴ however, it cannot be directly synthesized from PCho because phosphatidylethanolamine *N*-methyltransferase only interacts with the phosphatidylethanolamine substrate.

Adult major depression has been characterized in studies using 1.5-Tesla ^{31}P MRS by increases in PME peak (composed mainly of PCho and PEtn) in frontal regions⁷ but not in the basal ganglia.⁸ However, the composition of the PMEs peak at 1.5 Tesla does include glycerol-3-phosphate and inositol phosphates, leaving some uncertainty as to which actual metabolite is increased in depression. Proton MRS findings in late-life depression include increased choline resonance (PCho and GPCho) in the basal ganglia⁹ and reduced choline in the prefrontal cortex.¹⁰

Late-life depression has been hypothesized to have a vascular component,^{11,12} yielding a condition characterized by lower cardiac health,¹³ impaired executive function,^{14,15} psychomotor retardation and apathy,¹⁶ and treatment resistance to some antidepressants.^{17,18} Magnetic resonance imaging findings in late-life depression include increased white matter hyperintensities¹⁹ that are ischemic in nature²⁰ with concomitant microglial activation,²¹ demyelination,²² and other evidence of neuroinflammation. The volume of these ischemic lesions in patients with late-life depression significantly correlates with cognitive performance.²³ Reduced fractional anisotropy, as measured by diffusion tensor imaging,²⁴ has also been observed in patients with late-life depression. Taken together, these findings suggest that in late-life depression membrane integrity, particularly in white matter, may be compromised.

There are very large differences in the metabolic, functional, and neurodegenerative properties of gray and white matter.²⁵ Statistical methods have been developed that can incorporate information based on the segmentation of gray matter, white matter, and cerebrospinal fluid (CSF) into the analysis of three-dimensional chemical shift imaging (3D-CSI) ³¹P-MRS.²⁶⁻²⁸ Making use of these methods, hypotheses unique to specific tissue classes, and relating to the metabolic differences between them, can be tested over the whole brain.

We performed a 3D-CSI ³¹P MRS study at a 4-Tesla magnetic field strength measuring individual membrane metabolites in subjects with late-life depression versus normal comparison subjects. The following hypotheses were tested: 1) that the PDEs, GPCCho, and/or GPEtn in white matter will be increased in late-life major depression compared with normal elderly subjects, reflecting increased membrane breakdown in the white matter, and 2) that patients with late-life major depression will show an increase in PEtn and/or PCho, confirming the previous finding that PME was increased in major depression and clarifying which metabolite(s) are involved. A third hypothesis stated that PMEs would predict PDE metabolite levels and that these predictions would follow results from the known metabolic pathways for synthesis and degradation of membrane phospholipids, phosphatidylcholine and phosphatidylethanolamine.

METHODS

Subjects

The Institutional Review Board at McLean Hospital approved this study. Subjects were 21 older adults (ages 56–84) with either late-life depression (mean age $\pm$ standard deviation [SD]: 68.7 ± 9.29 ; 6 women and 4 men) or no history of psychiatric illness (mean age $\pm$ SD: 70.00 ± 8.33 ; 7 women and 4 men). Subjects were recruited from the Geriatric Psychiatry Program, McLean Hospital, Massachusetts General Hospital, Harvard Co-operative Project on Aging, or from the local community through advertisements. Members of the late-life depression group were diagnosed with *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*, Major Depressive Disorder and had a Hamilton Depression Scale-17 score ≥ 18 at the time of entry into the study. The members of the depression group did not have other Axis I disorders, unstable medical illness, untreated thyroid condition, or a history of substance abuse within the past year. Normal elderly subjects were accountable to the same exclusions as the depression group with the additional requirement that they not have any previous or current Axis I disorder and a Hamilton Depression Scale-17 score ≤ 8 . All subjects were free of benzodiazepines and were either not taking antidepressant medication or were nonresponsive to their medication, tapered off, and medication free (14 days MAOI; 7 days all others) at the time of scan.

The test–retest cohort included six neurologically and psychiatrically healthy adults (mean age $\pm$ SD: 35.7 ± 4.4 years; 3 women and 3 men) scanned on two separate visits, with follow-up Scan 2 completed 5.9 ± 0.9 weeks after Scan 1. All subjects were free of Axis I diagnoses, neurologic illness, severe medical problems, and psychoactive substance use. Exclusion criteria for all subjects included diagnoses of substance dependence, history of organic mental disorder, head trauma, loss of consciousness, seizure disorder or central

nervous system disease (e.g., multiple sclerosis or cerebral vascular incident), or contraindications to MR scanning (e.g., metallic implants, pacemaker, aneurysm clips, pregnancy or claustrophobia).

³¹P MRS Acquisition, Processing, and Analysis

For a detailed description of our methods for ³¹P-CSI data collection, processing, analysis, and segmentation, please refer to our previous publications.^{28,29} Briefly, a dual-tuned proton-phosphorus TEM head coil (Bioengineering Inc., Minneapolis, MN) was used for all anatomic imaging and spectroscopy. Manual shimming on the unsuppressed global water signal yielded a typical unsuppressed water line width of 20–30 Hz. High-contrast, T1-weighted sagittal and axial image sets of the entire brain were acquired using a three-dimensional, magnetization-prepared FLASH imaging sequence, allowing for clear segmentation between gray matter, white matter, and CSF as described in our previous publications.^{28,29} ³¹P 3D-CSI was used, allowing for the sampling of spatially resolved phosphorus spectra throughout the entire brain. In this study, we constrain our voxels to a 3 × 4 × 7 voxel submatrix, which samples most of the brain regions and contains the highest quality spectra (Fig. 2).

For ³¹P 3D-CSI spectral fitting, we used a spectral time-domain fitting program, based on the Marquardt-Levenberg nonlinear, least-squares algorithm, incorporating prior knowledge of spectral peak assignments, chemical shifts, and J-coupling constants.³⁰ Our spectral model included 10 phosphorus-containing molecules: gamma-, alpha-, beta-NTP, PEtn, PCho, GPEtn, GPCho, 2,3-diphosphoglyceride, inorganic phosphate, membrane-bound phospholipid, and PCr. The individual constituents for the PME and PDE regions (PEtn, PCho, GPEtn, GPCho) were all modeled as Gaussian singlets because our 4-Tesla spectra are coupled and J-coupled dispersion still exists within each one of these phospholipid resonances, thus affecting the line shape (Fig. 3). By combining the 3D-CSI data with segmented axial T1-weighted imaging data, we were able to relate ³¹P metabolite level to tissue content throughout the brain as described in detail in our previous publications.^{28,29}

Statistical Analysis

Test–Retest—Test–retest reliability was quantified using the coefficient of variation of the mean metabolite of interest to total signal (minus metabolite of interest) ratio across the 84 voxels for each scan. PCho, PEtn, GPCho, and GPEtn spectra were collected and quantified using an identical 3D-CSI protocol as was used in the study patients. The mean time between test and retest scans was 41 ± 7 days (mean ± SD). Because the SDs of the mean metabolite ratios appeared to increase as the means increased, the coefficients of variation were calculated using log-transformed values as previously recommended.³¹

Mixed Effects Models—Comparison of metabolite levels between subjects with late-life depression and comparison subjects was performed as described previously.²⁸ A linear mixed effects model was constructed for each of the four metabolites measured (GPCho, GPEtn, PCho, and PEtn) in brain. Each model included a random effect of subject and fixed effects of condition (depressed or normal comparison subject), partial volume (sum of percentages gray matter and white matter), gray–white matter difference, and total

phosphorous signal minus the metabolite of interest in the model as a normalizing covariate. Tissue composition and total phosphorous signal were calculated separately for each voxel, and model errors were assumed to be independent and identically distributed. Because one of our hypotheses involved examining metabolite levels between gray matter and white matter, we included interactions between condition and tissue type²⁸ that allow for the testing of these hypotheses. Continuous covariates were mean-centered so the main effect of condition quantified the mean change in metabolite concentration associated with depression for the average percentage of gray matter, white matter, and CSF observed for our sample. Additional candidate predictor variables (age and sex for PME and age, sex, PCho, and PEtn for PDE), followed by their interactions with our primary predictors, were added to the models if their associations with metabolite level were significant at the $\alpha = 0.05$ level without adjustment for multiple comparisons.

We tested for 1) differences in metabolite concentrations between depressed subjects and normal comparison subjects for the average amount of gray matter, white matter, and CSF observed in our sample (significance of the main effect of condition) and 2) associations of the differences between gray matter and white matter metabolite concentrations with depression (significance of the gray–white matter difference by condition) interaction. To aid in interpretation, we further estimated mean metabolite concentrations in the gray matter component and the white matter component of voxels of mixed composition from the model.²⁸ We performed a post-hoc significance test on the gray matter component and the white matter component if the interaction was significant.

Hypothesis tests were conducted using the Kenward-Roger adjustment, as recommended for small samples with unbalance in the number of repeated measures per individual.^{32,33} This method may result in noninteger valued denominator degrees of freedom for F statistics. Bonferroni corrections were applied separately for PMEs and PDEs to account for our choice of two outcomes in each category; therefore, all our p values were multiplied by 2, and the adjusted p value required for significance was 0.05. Linear models were fitted with the restricted maximum likelihood method used by JMP release 7 (SAS, Cary, NC).

RESULTS

Comparison subjects and depressed patients did not significantly differ in either sex or age. Cumulative Illness Rating Scale–Global total scores were available for 8 of the 11 normal elderly and for 9 of the 11 late-life depression patients and did not differ between groups (Student's $t(14) = 0.61$; $p = 0.55$). Coefficients of variation from test–retest data for the four metabolites examined in this study were 22% for PCho, 4% for PEtn, 14% for GPEtn, and 9% for GPCho.

Phosphomonoesters

Model building for the two PMEs, assessed in this study, PEtn and PCho, resulted in models that did not need correction for age or sex. For both PMEs, the area under the curve did not differ between late-life depression and normal elderly for voxels of average tissue content (Table 1). There were no significant effects of condition in gray and white matter (no

significant condition by tissue type interactions) for PCho ($F_{1, 1253} = 3.49$; $p = 0.12$; Fig. 4A) or PEtn ($F_{1, 1362} = 0.03$; $p = 1.0$; Fig. 4B).

Phosphodiesterases

Model building for GPCho area under the curve resulted in a final model that was not adjusted for age or sex. GPCho did not differ between late-life depression and normal elderly for voxels of average tissue content ($F_{1, 18.65} = 0.08$; $p = 1.0$; Table 1). There was no significant interaction between condition and tissue type ($F_{1, 1343} = 0.38$; $p = 1.0$) (Fig. 4C). When included in the model, PCho ($F_{1, 1212} = 18.32$; $p < 0.001$) and PEtn ($F_{1, 1205} = 36.93$; $p < 0.001$) both were significant predictors of GPCho (Fig. 5B, D). No interactions between these PME and depressed state or tissue type were observed.

GPEtn did not show a significant main effect of condition ($F_{1, 19} = 3.89$; $p = 0.12$; Table 1). However, there was a significant gray–white matter interaction with condition ($F_{1, 1292} = 7.94$; $p = 0.01$) such that white matter in subjects with late-life depression was elevated beyond levels seen in normal elderly ($t_{169.4} = -3.23$; $p = 0.003$) and gray matter GPEtn trended lower ($t_{830.9} = 2.07$; $p = 0.08$) in subjects with late-life depression compared with normal elderly (Fig. 4D). PEtn was a significant predictor of GPEtn ($F_{1, 1275} = 27.57$; $p < 0.001$), but PCho levels did not predict GPEtn levels ($F_{1, 1185} = 1.45$; not significant) (Fig. 5A, C). We observed an interaction between PEtn and tissue type ($F_{1, 1277} = 8.21$; $p = 0.008$), indicating a significant and observable influence of PEtn on phosphatidylethanolamine metabolism in gray matter ($t_{1278} = 3.76$; $p < 0.001$) when compared with white matter where there was no observable influence ($t_{1280} = -0.53$; $p = 1.0$; Fig. 6).

DISCUSSION

The major findings in this study are that, in accordance with our hypothesis, in late-life depression GPEtn was elevated in white matter when compared with normal comparison subjects. In late-life depression, GPEtn was found to trend lower in gray matter than it was in our normal elderly group. There was no significant difference in GPCho between late-life depression and normal elderly, and, unlike GPEtn, GPCho was significantly higher in white matter than in gray matter across all subjects, regardless of depressed state. Contrary to our hypothesis, we were not able to find a significant difference between normal elderly and late-life depression patients in either PME (PCho and PEtn) examined in this study. However, we were able to demonstrate that PMEs were highly significant predictors of the levels of PDEs, with two notable exceptions. PEtn was a significant overall predictor of GPEtn, but this effect could be entirely explained by its influence in gray matter but not in white matter, and PCho was not a significant predictor of the level of GPEtn.

Several features of phospholipid metabolism as well as inflammatory and neurodegenerative processes are likely influencing the results seen in this study. Phospholipids, including phosphatidylcholine and phosphatidylethanolamine, are the major constituents of cell membranes. Although in most mammalian tissues phosphatidylcholine is the predominant phospholipid, in the brain, the relative concentrations of phosphatidylcholine and phosphatidylethanolamine are essentially equal, with each comprising approximately 45%

of the total phospholipid pool.¹ Phosphatidylcholine and phosphatidylethanolamine are distributed asymmetrically across the neural plasma membrane,^{34,35} with phosphatidylcholine predominating on the extracellular leaflet of the plasma membrane and phosphatidylethanolamine predominating on the intracellular leaflet.

Phosphatidylcholine and phosphatidylethanolamine are both deacylated by phospholipase A₂ (PLA₂)² into lysophosphatidylcholine and lysophosphatidylethanolamine, which is subsequently transformed via the action of lysophospholipase into GPCCho and GPEtn.³⁶ Membranes are maintained in a homeostatic equilibrium between synthesis and degradation by the action of a calcium independent PLA₂ (iPLA₂).^{2,37-39} Increasing membrane precursor availability, either by adding exogenous precursor or by increasing the efficiency of the cytidine diphosphate–PCho citydyltransferase, has been noted to increase the quantity of GPCCho or GPEtn in cell culture³⁷ and in healthy tissue.^{2,3}

We observed substantially increased GPEtn in white matter in patients with late-life depression. Although this could be a sign of increased membrane turnover in these subjects, increased membrane turnover in the white matter would likely be accompanied by dependence of GPEtn levels on PEtn via the action of iPLA₂. However, this was not the case and suggests that other phospholipases, in particular cytosolic PLA₂ (cPLA₂), could be playing an active role in the observed increase in GPEtn in white matter. cPLA₂, in contrast to iPLA₂, releases arachidonic acid, initiating the release of a cascade of prostanoid chemokines and other eicosanoids⁴⁰ in activated microglia and astrocytes⁴¹ as well as neurons.⁴² In white matter, this inflammatory response could be initiated by myelin breakdown or other insults.^{42,43}

There is some support for this interpretation from studies of patients with bipolar disorder. Unmedicated, euthymic bipolar patients have been shown to have lower PME and higher PDE in the frontal lobe than normal comparison subjects, suggesting some loss of PME control of membrane turnover in these subjects.⁴⁴ In addition, an up-regulation of the arachidonic acid cascade through activity of cPLA₂ has been observed postmortem from bipolar disorder cases,⁴⁵ whereas medications that are effective as mood stabilizers down-regulate the arachidonic acid cascade.⁴⁶ Therefore, the loss of dependence of GPEtn on PME levels suggests a mechanism not based on membrane turnover and is possibly a sign of increased membrane degeneration and inflammation in white matter in late-life depression compared with normal elderly.

Depression in late life is associated with the presence of cardiovascular illness⁴⁷ and factors that increase the vascular risk profile, including diabetes,⁴⁸ smoking,⁴⁹ and hypertension.⁵⁰ A prominent finding in late-life depression is an increase in subcortical white matter hyperintensities²³ and decreases in fractional anisotropy²⁴ even when vascular risk factors are controlled. Further studies looking at the relationship between other indicators of white matter degeneration, such as fractional anisotropy, and GPEtn in white matter could help to clarify the meaning of this finding.

There is no significant difference in white matter GPCCho between normal and late-life depression subjects; however, both PCho and GPCCho are substantially higher in white

matter compared with gray matter in all subjects. This difference is not seen in GPEtn, suggesting a difference in membrane composition between the two tissue types, perhaps resulting from the incorporation of GPCho cholinephosphodiesterase in myelin.⁵¹

One of the main limitations of this study is the small sample size used. Although the 3D-CSI method, yielding 84 voxels per subject, combined with the mixed effects models used to analyze the data generates substantial power, a lack of statistical significance should be interpreted cautiously. A second limitation is that our study did not specifically select for patients with late-onset depression; therefore, our late-life depression sample was a heterogeneous mix of patients with variable depression histories. A note of caution is also warranted due to the inherently low signal-to-noise ratio of the PCho resonance. Our measured coefficient of variation in the test—retest study is somewhat high at 22%. Therefore, it is possible that the low signal-to-noise ratio could have affected our finding that PCho predicted GPCho and not GPEtn. However, because PCho prediction of GPCho was strong, it is unlikely that low signal-to-noise could entirely account for the lack of prediction of GPEtn.

Finally, we did not make any attempt to provide regional analysis of our data. We made this choice, first, due to the large size of ³¹P voxels that were used and, second, because our white matter hypothesis was relevant to the whole brain and our sample size small, we would unacceptably increase the probability of Type II error, masking important results. Future studies, with a larger sample size and using a different ³¹P MRS sampling scheme generating smaller voxels, may be able to allow regional hypotheses to be tested.

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References

1. Vance JE. Phosphatidylserine and phosphatidylethanolamine in mammalian cells: two metabolically related aminophospholipids. *J Lipid Res.* 2008; 49:1377–1387. [PubMed: 18204094]
2. Baburina I, Jackowski S. Cellular responses to excess phospholipid. *J Biol Chem.* 1999; 274:9400–9408. [PubMed: 10092620]
3. Babb SM, Wald LL, Cohen BM, et al. Chronic citicoline increases phosphodiesters in the brains of healthy older subjects: an in vivo phosphorus magnetic resonance spectroscopy study. *Psychopharmacology (Berl).* 2002; 161:248–254. [PubMed: 12021827]
4. Kennedy EP, Weiss SB. The function of cytidine coenzymes in the biosynthesis of phospholipides. *J Biol Chem.* 1956; 222:193–214. [PubMed: 13366993]
5. Bremer J, Figard PH, Greenberg DM. The biosynthesis of choline and its relation to phospholipid metabolism. *Biochim Biophys Acta.* 1960; 43:477–488.

6. Blusztajn JK, Zeisel SH, Wurtman RJ. Synthesis of lecithin (phosphatidylcholine) from phosphatidylethanolamine in bovine brain. *Brain Res.* 1979; 179:319–327. [PubMed: 509240]
7. Volz HP, Rzanny R, Riehemann S, et al. ³¹P magnetic resonance spectroscopy in the frontal lobe of major depressed patients. *Eur Arch Psychiatry Clin Neurosci.* 1998; 248:289–295. [PubMed: 9928907]
8. Moore CM, Christensen JD, Lafer B, et al. Lower levels of nucleoside triphosphate in the basal ganglia of depressed subjects: a phosphorous-31 magnetic resonance spectroscopy study. *Am J Psychiatry.* 1997; 154:116–118. [PubMed: 8988971]
9. Chen CS, Chiang IC, Li CW, et al. Proton magnetic resonance spectroscopy of late-life major depressive disorder. *Psychiatry Res.* 2009; 172:210–214. [PubMed: 19303260]
10. Venkatraman TN, Krishnan RR, Steffens DC, et al. Biochemical abnormalities of the medial temporal lobe and medial prefrontal cortex in late-life depression. *Psychiatry Res.* 2009; 172:49–54. [PubMed: 19179054]
11. Alexopoulos GS, Meyers BS, Young RC, et al. “Vascular depression” hypothesis. *Arch Gen Psychiatry.* 1997; 54:915–922. [PubMed: 9337771]
12. Krishnan KR, Hays JC, Blazer DG. MRI-defined vascular depression. *Am J Psychiatry.* 1997; 154:497–501. [PubMed: 9090336]
13. Alexopoulos GS, Kiosses DN, Murphy C, et al. Executive dysfunction, heart disease burden, and remission of geriatric depression. *Neuropsychopharmacology.* 2004; 29:2278–2284. [PubMed: 15340393]
14. Alexopoulos GS, Kiosses DN, Klimstra S, et al. Clinical presentation of the “depression-executive dysfunction syndrome” of late life. *Am J Geriatr Psychiatry.* 2002; 10:98–106. [PubMed: 11790640]
15. Barnes DE, Alexopoulos GS, Lopez OL, et al. Depressive symptoms, vascular disease, and mild cognitive impairment: findings from the Cardiovascular Health Study. *Arch Gen Psychiatry.* 2006; 63:273–279. [PubMed: 16520432]
16. Krishnan KR, Taylor WD, McQuoid DR, et al. Clinical characteristics of magnetic resonance imaging-defined subcortical ischemic depression. *Biol Psychiatry.* 2004; 55:390–397. [PubMed: 14960292]
17. Alexopoulos GS, Kiosses DN, Choi SJ, et al. Frontal white matter microstructure and treatment response of late-life depression: a preliminary study. *Am J Psychiatry.* 2002; 159:1929–1932. [PubMed: 12411231]
18. Sheline YI, Pieper CF, Barch DM, et al. Support for the vascular depression hypothesis in late-life depression: results of a 2-site, prospective, antidepressant treatment trial. *Arch Gen Psychiatry.* 2008; 67:277–285. [PubMed: 20194828]
19. MacFall JR, Taylor WD, Rex DE, et al. Lobar distribution of lesion volumes in late-life depression: the Biomedical Informatics Research Network (BIRN). *Neuropsychopharmacology.* 2006; 31:1500–1507. [PubMed: 16341022]
20. Thomas AJ, O’Brien JT, Davis S, et al. Ischemic basis for deep white matter hyperintensities in major depression: a neuropathological study. *Arch Gen Psychiatry.* 2002; 59:785–792. [PubMed: 12215077]
21. Simpson JE, Ince PG, Higham CE, et al. Microglial activation in white matter lesions and non-lesional white matter of ageing brains. *Neuropathol Appl Neurobiol.* 2007; 33:670–683. [PubMed: 17990995]
22. Simpson JE, Fernando MS, Clark L, et al. White matter lesions in an unselected cohort of the elderly: astrocytic, microglial and oligodendrocyte precursor cell responses. *Neuropathol Appl Neurobiol.* 2007; 33:410–419. [PubMed: 17442062]
23. Sheline YI, Price JL, Vaishnavi SN, et al. Regional white matter hyperintensity burden in automated segmentation distinguishes late-life depressed subjects from comparison subjects matched for vascular risk factors. *Am J Psychiatry.* 2008; 165:524–532. [PubMed: 18281408]
24. Shimony JS, Sheline YI, D’Angelo G, et al. Diffuse microstructural abnormalities of normal-appearing white matter in late life depression: a diffusion tensor imaging study. *Biol Psychiatry.* 2009; 66:245–252. [PubMed: 19375071]

25. Erecinska M, Silver IA. ATP and brain function. *J Cereb Blood Flow Metab.* 1989; 9:2–19. [PubMed: 2642915]
26. Hetherington HP, Spencer DD, Vaughan JT, et al. Quantitative (31)P spectroscopic imaging of human brain at 4 Tesla: assessment of gray and white matter differences of phosphocreatine and ATP. *Magn Reson Med.* 2001; 45:46–52. [PubMed: 11146485]
27. Mason GF, Chu WJ, Vaughan JT, et al. Evaluation of ³¹P metabolite differences in human cerebral gray and white matter. *Magn Reson Med.* 1998; 39:346–353. [PubMed: 9498589]
28. Forester BP, Harper DG, Jensen JE, et al. (31)Phosphorus magnetic resonance spectroscopy study of tissue specific changes in high energy phosphates before and after sertraline treatment of geriatric depression. *Int J Geriatr Psychiatry.* 2009
29. Forester BP, Berlow YA, Harper DG, et al. Age-related changes in brain energetics and phospholipid metabolism. *NMR Biomed.* 2010; 23:242–250. [PubMed: 19908224]
30. Jensen JE, Drost DJ, Menon RS, et al. In vivo brain (31)P-MRS: measuring the phospholipid resonances at 4 Tesla from small voxels. *NMR Biomed.* 2002; 15:338–347. [PubMed: 12203225]
31. Rosner, B. *Fundamentals of Biostatistics.* 5. New York: Duxbury Press; 2000.
32. Kenward MG, Roger JH. Small sample inference for fixed effects from restricted maximum likelihood. *Biometrics.* 1997; 53:983–997. [PubMed: 9333350]
33. Kowalchuk RK, Keselman HJ, Algina J, et al. The analysis of repeated measurements with mixed-model adjusted F tests. *Educ Psychol Measur.* 2004; 64:224–242.
34. Ikeda M, Kihara A, Igarashi Y. Lipid asymmetry of the eukaryotic plasma membrane: functions and related enzymes. *Biol Pharm Bull.* 2006; 29:1542–1546. [PubMed: 16880601]
35. Daleke DL. Phospholipid flippases. *J Biol Chem.* 2007; 282:821–825. [PubMed: 17130120]
36. Morash SC, Cook HW, Spence MW. Lysophosphatidylcholine as an intermediate in phosphatidylcholine metabolism and glycerophosphocholine synthesis in cultured cells: an evaluation of the roles of 1-acyl- and 2-acyl-lysophosphatidylcholine. *Biochim Biophys Acta.* 1989; 1004:221–229. [PubMed: 2752020]
37. Morash SC, Cook HW, Spence MW. Phosphatidylcholine metabolism in cultured cells: catabolism via glycerophosphocholine. *Biochim Biophys Acta.* 1988; 961:194–202. [PubMed: 3390456]
38. Balsinde J, Balboa MA. Cellular regulation and proposed biological functions of group VIA calcium-independent phospholipase A₂ in activated cells. *Cell Signal.* 2005; 17:1052–1062. [PubMed: 15993747]
39. Barbour SE, Kapur A, Deal CL. Regulation of phosphatidylcholine homeostasis by calcium-independent phospholipase A₂. *Biochim Biophys Acta.* 1999; 1439:77–88. [PubMed: 10395967]
40. Ghosh M, Tucker DE, Burchett SA, et al. Properties of the group IV phospholipase A₂ family. *Prog Lipid Res.* 2006; 45:487–510. [PubMed: 16814865]
41. Rao JS, Kellom M, Kim HW, et al. Neuroinflammation and synaptic loss. *Neurochem Res.* 2012
42. Palumbo S, Toscano CD, Parente L, et al. Time-dependent changes in the brain arachidonic acid cascade during cuprizone-induced demyelination and remyelination. *Prostagl Leukot Essent Fatty Acids.* 2011; 85:29–35.
43. Clarner T, Diederichs F, Berger K, et al. Myelin debris regulates inflammatory responses in an experimental demyelination animal model and multiple sclerosis lesions. *Glia.* 2012
44. Deicken RF, Fein G, Weiner MW. Abnormal frontal lobe phosphorous metabolism in bipolar disorder. *Am J Psychiatry.* 1995; 152:915–918. [PubMed: 7755123]
45. Kim HW, Rapoport SI, Rao JS. Altered arachidonic acid cascade enzymes in postmortem brain from bipolar disorder patients. *Mol Psych.* 2011; 16:419–428.
46. Rao JS, Rapoport SI. Mood-stabilizers target the brain arachidonic acid cascade. *Curr Mol Pharmacol.* 2009; 2:207–214. [PubMed: 20021459]
47. Carney RM, Blumenthal JA, Stein PK, et al. Depression, heart rate variability, and acute myocardial infarction. *Circulation.* 2001; 104:2024–2028. [PubMed: 11673340]
48. Lustman PJ, Griffith LS, Gavard JA, et al. Depression in adults with diabetes. *Diabetes Care.* 1992; 15:1631–1639. [PubMed: 1468296]
49. Trosclair A, Dube SR. Smoking among adults reporting lifetime depression, anxiety, anxiety with depression, and major depressive episode. United States, 2005–2006. *Addict Behav.* 2009

50. Rutledge T, Hogan BE. A quantitative review of prospective evidence linking psychological factors with hypertension development. *Psychosom Med.* 2002; 64:758–766. [PubMed: 12271106]
51. Sok DE, Kim MR. Brain myelin-bound Zn(2+)-glycerophosphocholine cholinephosphodiesterase is a glycosylphosphatidylinositol-anchored enzyme of two different molecular forms. *Neurochem Res.* 1994; 19:97–103. [PubMed: 8139771]

FIGURE 1.

Biosynthetic pathways of phosphatidylcholine and phosphatidylethanolamine (Kennedy pathways). *CT*, CTP: phosphocholine cytidyltransferase; *ET*, CTP: phosphoethanolamine cytidyltransferase; *CPT*, CDP-choline:1, 2-diacylglycerol cholinephosphotransferase; *PEMT*: phosphatidylethanolamine N-methyltransferase; *PTSD*: phosphatidylserine decarboxylase; *PSS*: phosphatidylserine synthase; *PLA₂*: phospholipase A₂; *LPL*: lysophospholipase.

FIGURE 2.

T1-weighted axial [A] and sagittal [B] anatomic images depicting the placement of the $4 \times 7 \times 3$ CSI submatrix inside the brain. Using the axial images, the submatrix was centered along the central sulcus (left–right) and in the anterior–posterior dimension. Using the sagittal images, the submatrix was positioned such that the superior boundary of the middle slice lined up with the superior-most point of the corpus callosum. The effective volume of each voxel after processing is approximately 25 mL. The submatrix contains primarily the thalamic–basal ganglia anatomy as well as portions of the temporal, parietal, and frontal cortices and intervening white matter.

FIGURE 3.

A high signal-to-noise in vivo phosphorus spectrum from nine summed voxels clearly displays the resolved resonances in the PME region (PEtn, phosphoserine [PSer], and PCho) as well as in the PDE region (GPEtn, GPCho, and membrane-bound phospholipid [MP]). The raw spectrum is displayed with 5 Hz exponential filtering for display [B] and is included with modeled fit [C] and residual [A]. In [D] the individual modeled spectral components are displayed for each fitted resonance. The modeled resonances for 2,3-diphosphoglycerate (2,3-DPG) are not labeled for display purposes and exist between PCho and inorganic phosphate (Pi).

FIGURE 4.

Least-squares means ($\pm$ 95% confidence interval) adjusted for total phosphorus signal in both gray matter and white matter levels of [A] PCho, [B] PEtn, [C] GPCho, and [D] GPEtn. There were no significant model main effects for comparison subjects versus depressed. However, there was a significant main effect for tissue type in [A] PCho ($F_{1, 1253} = 5.44$; $p = 0.04$) largely, but not significantly, accounted for by the gray–white matter difference in normal elderly subjects. A significant tissue type difference in [C] GPCho ($F_{1, 1343} = 31.6128$; $p < 0.0001$) was seen equally in both groups. No effects were noted for [B] PEtn. A significant tissue type interaction was noted in [D] GPEtn between normal elderly and late-life depression patients ($F_{1, 1292} = 7.94$; $p = 0.01$) that was largely accounted for by an increase in white matter GPEtn in the depressed subjects compared with normal comparison subjects ($t_{169} = -3.23$; $p = 0.003$).

FIGURE 5.

Model-derived prediction of PEtn on levels of [A] GPEtn and [C] GPCho and PCho on [B] GPEtn and [D] GPCho in the combined population of normal elderly and late-life depression patients. Black lines indicate the least-squares prediction, dashed blue lines indicate the 95% confidence interval, and dashed red lines indicate the centered mean. If the 95% confidence interval travels outside the centered mean, then the result is significant. PEtn levels significantly predicted levels of GPEtn ($F_{1,1275} = 27.57$; $p < 0.0001$) and GPCho ($F_{1,1205} = 36.92$; $p < 0.0001$), whereas PCho predicted GPCho levels ($F_{1,1212} = 18.3181$; $p < 0.0001$) but did not predict GPEtn ($F_{1,1185} = 1.45$; $p = 0.2290$) in agreement with predictions based on the Kennedy pathways and the action of phosphatidylethanolamine-n-methyl transferase (see Fig. 6).

FIGURE 6.

Prediction of GPEtn by PEtn was found to vary by tissue type (gray versus white matter). In gray matter, PEtn significantly predicted GPEtn ($t_{1278} = 3.76$; $p = 0.0002$), whereas in white matter there was no significant prediction ($t_{1280} = -0.52$; $p = 0.6$).

PME and PDE Least-Squares Mean Estimates of Area Under the Curve for Model Main Effects (an Average Voxel Containing an Average Amount of Gray Matter, White Matter, and CSF)

TABLE 1

Metabolite × 100	Control Subjects	Depressed Patients		Comparison		
	Mean ± 95% CI	Mean ± 95% CI	df	F	p	
Phosphomonoesters						
PEtn	1.590 ± 0.075	1.635 ± 0.079	1, 18.8	0.73	0.81	
PCho	0.795 ± 0.046	0.845 ± 0.048	1, 20.2	2.14	0.32	
Phosphodiester						
GPCho	1.672 ± 0.073	1.665 ± 0.077	1, 18.7	0.08	1.0	
GPEtn	1.120 ± 0.061	1.205 ± 0.063	1, 19.1	3.89	0.92	

Notes: Hypothesis tests used the Kenward-Roger adjustment, which may result in noninteger valued degrees of freedom. There were no significant differences between normal elderly and subjects with late-life depression in these model main effects.