Neuropsychopharmacology (2004) 29, 2251–2257

www.neuropsychopharmacology.org

Brain Metabolites and Cognitive Function among Older Depressed and Healthy Individuals Using 2D MR Spectroscopy

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Brain metabolites of choline (Ch) and *myo*-Inisotol (mI) have been reported as elevated among geriatric depressed patients. Two-dimensional (2D) magnetic resonance spectroscopy (MRS) provides estimates of Ch, mI, and creatine (Cr) similar to one-dimensional MRS, and it also estimates the resonances of the Ch-containing compounds of phosphoethanolamine (Pe) and phosphocholine (PCh). In this cross-sectional geriatric study, 14 depressed patients and 14 healthy volunteers who were comparable in age, gender, education, comorbid medical burden, and Mini-Mental State Examination (MMSE) scores completed 2D MRS and a neurocognitive battery. A voxel in the left dorsolateral cortex, which was comprised of approximately 60% white matter, was used to estimate the CR ratios of Ch, PCh, Pe, and mI. Composite scores for cognitive function were developed for verbal learning, recall, recognition, executive function, hypothesis generation, and processing speed. Among nondepressed subjects, cognition was positively correlated with Ch/Cr and mI/Cr and negatively correlated with PCh/Cr in four domains of verbal learning, recognition, recall, and hypothesis generation. In contrast, depressed patients did not have consistent relationships between Ch/Cr, mI/Cr, and PCh/Cr and cognition. There was a significant difference in the overall pattern of associations between the four metabolites and verbal learning and processing speed in depressed patients compared to healthy controls. The attenuated relationship between metabolites and specific cognitive domains in patients with late-life MDD suggests that the level of cognitive performance observed during depressive episodes may be associated with changes in biochemistry within the frontostriatal neuronal circuitry.
Neuropsychopharmacology (2004) **29,** 2251–2257, advance online publication, 8 September 2004; doi:10.1038/sj.npp.1300553

Keywords: neuroimaging; cognition; spectroscopy; depression; elderly; metabolites

INTRODUCTION

Neuronal circuits regulate and modulate emotional and cognitive functions in humans, primates, and less evolved animals (Fuster 2000, 2001; Goldman-Rakic 1995; Mesulam 2000). These circuits form the communicating pathways between the prefrontal cortex and the primary and associative processing regions of the brain (Fuster 2000, 2001; Price 2001), and impairment of these circuits are believed to contribute to mood and psychiatric disorders (Drevets and Raichle, 1992; Kumar and Cook, 2002; Thomas *et al*, 2002).

Magnetic resonance spectroscopy (MRS) is a neuroimaging technique that makes possible the examination of the biochemical correlates of mood, cognition, and related behavioral domains. Among elderly patients, there is growing evidence from one-dimensional (1D) MRS that the level of choline (Ch) is elevated during unipolar depression in the frontal cortex and basal ganglia (Charles *et al*, 1994; Hamakawa *et al*, 1998; Kumar *et al*, 2002; Steingard *et al*, 2000), and that it decreases with successful treatment (Renshaw *et al*, 1997). The Ch resonance also includes the compounds of glycerophosphocholine, phosphoethanolamine (Pe), and phosphocholine (PCh) (Miller, 1991), and it is not clear which of the Ch compounds becomes elevated with the onset of depression.

myo-Inisotol (mI) has also been reported as elevated in the frontal cortex among elderly depressed patients using 1D MRS (Kumar *et al*, 2002). It is involved in several important cellular processes in the brain as a marker of phospholipid turnover and gliosis, a secondary messenger and a detoxifying molecule (Catani *et al*, 2002; Lopez-Villegas *et al*, 1997; Shonk *et al*, 1995). In addition to depression, mI has been observed as elevated in patients with Alzheimer's disease, mild cognitive impairment (Huang *et al*, 1999; Kantarci *et al*, 2000; Kumar *et al*, 2002), primary progressive aphasia (Catani *et al*, 2003), and hypertension (Catani *et al*, 2002).

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Received 20 November 2003; revised 8 July 2004; accepted 13 July 2004

The goal of this study was to examine the relationships between cognition and the biochemical concentrations of mI and Ch across healthy and depressed geriatric volunteers. Two-dimensional (2D) MR spectroscopy is a new technique that, by virtue of a second spectral dimension, can differentiate the Pe and PCh resonances from the Ch resonance. Hence, in addition to Ch, we could examine how PCh and Pe are associated with cognitive performance and if the associations of the three metabolites are different across diagnostic groups. The mI resonance was examined to determine if its relationship to cognition also varied across groups and if it had a recognizable pattern *vis-à-vis* the Ch compounds. NAA was not a research focus due to the lack of support in the literature for an association between NAA levels and late-life depression (Kumar *et al*, 2002; Charles *et al*, 1994).

METHODS

Subjects

This study was conducted with approval from the Institutional Review Board at the University of California, Los Angeles, in accord with the Helsinki Declaration of 1975. In all, 28 community-dwelling volunteers, 14 depressed and 14 healthy, were recruited from surrounding communities with newspaper and radio announcements. The exclusion criteria included history of substance abuse per the Structured Clinical Interview for Diagnosis for Axis I disorders (SCID); clinical evidence of dementia; presence of neurological or endocrine disease such as diabetes, Parkinson's disease, or syphilis; history of head trauma; presence of a current unstable or serious medical illness; or presence of contraindicators for neuroimaging such as an implanted electronic device or skeletal prosthesis. Depressed patients met DSM-IV criteria for major depression, scored $\geqslant$15 on the 17-item Hamilton Rating Scale for Depression (Hamilton, 1960), and were drug free for antipsychotics, anxiolytics, and antidepressant medications for at least 2 weeks prior to inclusion in the study. The inclusion criteria included age $\geqslant$60 years of age, English-speaking, and hearing and visual ability sufficient to complete the full neuropsychological battery.

Study Design

Volunteers were screened for this cross-sectional study with the SCID, given a neurological examination by a clinical geriatric psychiatrist, and administered the Mini-Mental State Examination (MMSE) (Folstein *et al*, 1975) and the Cumulative Illness Rating Scale, a commonly used measure of medical comorbidity among geriatric patients (Linn *et al*, 1968). None of the participants had a history or mental status suggestive of dementia or any other brain disorder. Volunteers who passed the initial screening process completed an EKG and a standard battery of laboratory tests, including complete and differential blood counts; liver, renal, and thyroid screening tests; and measurement of electrolyte levels. Administration of a comprehensive neuropsychological battery and neuroimaging was completed within 2 weeks of the initial screening for depressed patients and within 4 weeks for the controls.

MRS Acquisition and Postprocessing

^{1}H MR spectra were acquired in the left dorsolateral prefrontal white matter region using a 1.5 T GE MRI/MRS scanner (GE Medical Systems, Waukesha, WI, USA), with a body coil for 'transmission' and a 3 in surface coil for 'reception'. The 3 in coil was placed directly on the forehead of the subject and a $3 \times 3 \times 3$ cm^3 voxel was positioned to the left side of the forehead, just above the eye to be closer to the left frontal white matter region. The 2D L-COSY sequence consisted of three slice-selective rf pulses (90°, 180°, and 90°) for the volume localization and the coherence transfer necessary for 2D-correlated spectroscopy (Thomas *et al*, 2003, 2001). A CHESS sequence was used for global water suppression prior to volume localization. 2D L-COSY spectra were recorded using the following parameters: TE = 30 ms, TR = 2 s, and the total number of scans of 800 (100 Δt_1 increments and 8 number of excitations per Δt_1), corresponding to a total duration of approximately 27 min, respectively, for each 2D scan. The 2D raw matrix consisted of 1024 complex points along the first dimension and 100 points along the second dimension. The matrices were zero filled to 2048×256, and processed using a Felix-2000 package (Accelrys, San Diego, CA, USA). The volumes under the 2D diagonal and crosspeaks were calculated using manual peak picking on contours. The area of integration for each peak was kept constant in all the measurements as reported recently (Binesh *et al*, 2002). The ratio of each metabolite peak volume was calculated with respect to the diagonal peak of Cr (Cr_d). Only the analysis of the four metabolites of mI, Pe, PCh, and Ch, which are the most likely to be associated with depression, and cognition are reported in this work (Figure 1).

We tested the reproducibility of 2D L-COSY spectra using phantom solutions and also, in a different location of adult human volunteers, the anterior cingulate gray matter (Binesh *et al*, 2002). In a total of 10 healthy volunteers, the following coefficients of variation of the ratios with respect to the diagonal peak of Cr were calculated: (a) Ch: 6.1%; (b) mI: 15%; (c) PCh: 26.1%; and (d) Pe: 21.3%. The coefficients of variance of the absolute 2D diagonal peak volumes of Cr and Ch were 19 and 20%, respectively.

The volumes under the diagonal peaks of creatine (Cr) and Ch were calculated at ($F_2 = F_1 = 3.0$ ppm) and ($F_2 = F_1 = 3.2$ ppm) (denoted as Cr_d and Ch_d, respectively). Four 2D crosspeaks were selected at the following locations: mI ($F_2 = 3.5$ ppm, $F_1 = 3.1$ ppm), Ch ($F_2 = 4.0$ ppm, $F_1 = 3.5$ ppm), Pe ($F_2 = 4.0$ ppm, $F_1 = 3.15$ ppm), and PCh ($F_2 = 4.3$ ppm, $F_1 = 3.7$ ppm). The peak volumes of crosspeaks above the diagonal were discarded due to the asymmetry effect (Banaker *et al*, 2002). The mI crosspeaks were identified at two locations, the first at $F_2 = 3.5$ ppm and $F_1 = 3.1$ ppm solely due to mI and the second at $F_2 = 4.0$ ppm and $F_1 = 3.5$ ppm overlapping with free Ch, so the former was used for this analysis. The PCh peak overlaps with glycerylphosphocholine, and the Pe peak overlaps with glycerophosphethanolamine. The assignments of these peaks were guided by the 2D L-COSY spectra of individual phantom solutions of several metabolites (Thomas *et al*, 2001) and a previous report on the 1D MRS chemical shifts (Govindaraju *et al*, 2000).

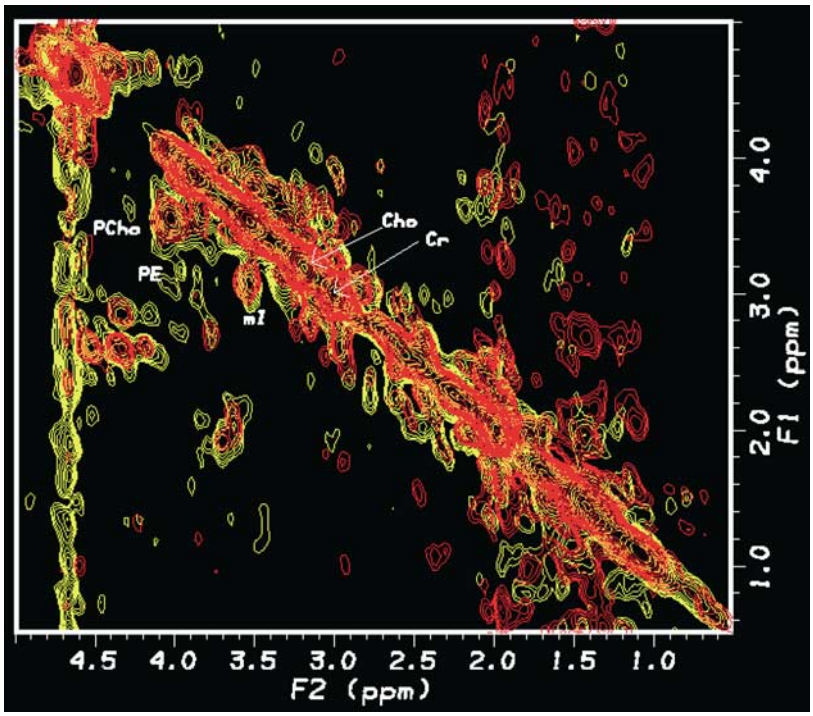

Figure 1 The spectra of a depressed patient (red) superimposed on a healthy control (yellow). (TE = 30 ms; TR = 2 s; 100 rows; eight averages). Intergroup *t*-tests showed no significant differences in metabolite levels.

MRI Methods

The volumes of intracranial gray matter, white matter, and CSF volumes in the MRS voxel were calculated from T_1-weighted, three-dimensional (3D) volumetric spoiled gradient recalled echo (SPGR) images acquired using the following parameters: 1.6-mm slice thickness, $22 \times 16.5\,cm^2$ field of view, 124 contiguous partitions, 192 views, and 45° flip angle (TE = 5 ms, TR = 42 ms). A conventional quadrature head coil was used for the volumetric MRI. The spectroscopy voxels ($3 \times 3 \times 3\,cm^3$) were localized on a T_1-weighted axial MRI recorded using a 3 in surface 'receive' coil acquired at the same scanning session as the 3D SPGR data. Segmentation of the 3D MRI data was accomplished with a minimum distance classifier algorithm (Kollokian, 1996), which has been previously described (Kumar *et al*, 2002).

Neuropsychological Battery

The neuropsychological tests were selected to characterize six neuropsychological domains that have been associated with the left lateral cortex by lesion analysis and neuroimaging: verbal learning, verbal recall, verbal recognition, verbal executive function, hypothesis generation, and processing speed. Loading of test variables on domain scales was based on an *a priori* assessment of content validity, which has been used by others (Bilder *et al*, 2000; Cannon *et al*, 1994) and which has been demonstrated in a principle component analysis with a larger sample (Blanchard and Neale, 1994; Elderkin-Thompson *et al*, 2003a).

The domain scales and contributing tests were as follows: *verbal learning*: California Verbal Learning Test (CVLT) (Delis *et al*, 1987), trials 1–5 inclusive, trial 1, and trial 5; *verbal recall:* CVLT short-delayed free recall and long-delayed free recall; *verbal recognition*: CVLT recognition, Recognition Memory Test for Words (RMT) (Warrington, 1984); *verbal executive function:* Stroop Interference Trial (Stroop, 1935), Trailmaking Part B (Lezak, 1995); *hypothesis generation:* Wisconsin Card Sort Test (WCST) (Heaton *et al*, 1993) conceptual responses and categories achieved; and *processing speed:* WAIS-III Digit Symbol (Wechsler, 1997), Stroop 1 (Color Naming) and Stroop 2 (Word Reading–Kaplan version);(Mitrushina *et al*, 1999), and Trailmaking Part A (Lezak, 1995).

Data Analysis

Sample characteristics were examined with Student's *t*-tests for continuous variables and χ^2 for nominal variables. Scatterplots of cognitive test scores were examined for outliers >2 SD before scores were converted to *z* scores using the mean and standard deviation of the healthy comparison group. Composite scale scores for each domain were computed by averaging *z* scores of contributing variables per the *a prior* protocol. All scales were computed so that higher scores indicated better performance. The spectroscopy estimates of the absolute contour volumes of mI, Ch, Pe, PCh, and Cr were used to determine the ratios. Even though Cr tends to increase over the lifespan (Chang *et al*, 1996; Pfefferbaum *et al*, 1999), within a limited age span as represented in this sample, the CR is likely to be sufficiently stable to allow for comparisons between the diagnostic groups. *T*-tests were computed to confirm that Cr levels were comparable between groups (controls = 670.14 ($\pm$131.04 SD) and patients = 689.36

Table 1 Demographic and Clinical Information of Patients and Controls

	Depressed patients ($N=14$)		Controls ($N=14$)		
	Means (SD)	**Range**	**Mean (SD)**	**Range**	***p*-value**
Age (years)	70.6 (±6.4)	61–86	70.4 (±6.7)	60–84	0.93
Sex: male/female	3/11		3/11		0.68
Years education	14.5 (±2.2)	12–20	15.9±(2.8)	11–20	0.16
MMSE score	29.2 (±1.7)	25–30	29.2 (±1.3)	26–30	1.00
Handedness: right/left	13/1		13/1		0.76
Ethnicity					
Caucasian	13		11		0.54
African-American			1		
Asian			1		
Other	1		1		
HAM-D	17.4 (±3.0)	15–25	NA	NA	
CIRS categories	3.4 (±1.4)	1–6	2.4 (±1.6)	1–6	0.12

NA = not applicable.

(±105.27 SD), $p=0.67$) and between young (60–69 years, $N=13$) and old (70–90 years, $N=15$) subjects ($t(1.299)=0.21$, df 26) before ratios were computed using Cr as the denominator.

Multiple regression analyses were used to examine how efficiently the metabolites predicted cognitive function in the depressed and control groups, and to assess whether the four-metabolite model differed across groups. In the separate univariate regression analyses, the dependent variables were the composite neuropsychological scores, and the independent variables were the four metabolites, diagnosis (a dummy variable coded 1 = depressed and 0 = control), and the interaction terms (each metabolite × diagnosis). Education was entered as a covariate. The interaction terms showed the differences between the two diagnostic groups in the slope of each of the four metabolites for each dependent variable. Planned contrasts were performed that tested the overall predictive models within each group, and tested the significance of the overall group differences for the model as a whole (both of these tests have numerator df = 4) (Table 1).

RESULTS

The standardized regression coefficients for each diagnostic group and the difference scores are displayed in Table 2. Overall, the four metabolites accounted for 34 to 55% of the unexplained variance in the composite cognition scales. Among the healthy participants, the four-metabolite model successfully predicted Verbal Learning and Verbal Recognition scores, with Hypothesis Generation and Processing Speed scores showing a trend toward significance ($p=0.02$, 0.03, 0.18, and 0.14, respectively). Among depressed patients, the model predicted scores on the Processing Speed scale ($p=0.04$), but it did not predict any of the other domains. Difference analysis indicated that the metabolite models differed significantly between healthy and depressed groups in Verbal Learning and Processing Speed ($p=0.02$ and 0.03, respectively).

In terms of the individual metabolites, the Ch/Cr ratios of healthy controls were significant and positive predictors of Verbal Learning, Verbal Recall, Verbal Recognition and Hypothesis Generation scales ($p=0.02$, 0.04, 0.02, and 0.04, respectively). mI/Cr significantly predicted Verbal Learning ($p=0.01$) and Verbal Recognition ($p<0.01$) and had moderate but nonsignificant coefficients in the remaining two composite scores of Verbal Recall and Hypothesis Generation ($p=0.19$ and 0.11, respectively). In contrast, negative coefficients emerged between PCh/Cr and the same four composite scales, with a significant coefficient for Verbal Learning ($p=0.05$). In contrast, the depressed group showed no pattern of associations between the cognitive domains and the metabolites. Only one individual relationship was significant, the mI/Cr association with Processing Speed.

The differences in regression coefficients (ie, the interaction terms) are computed by subtracting the coefficients of controls from those of patients in planned contrasts. Negative values indicate a greater positive slope for controls than for depressed patients. Thus, cognitive performance of controls improved to a greater degree as the volume of Ch/Cr and mI/Cr increased than it did among depressed persons. The difference between the slopes reached significance for the Ch/Cr and mI/Cr volumes in Verbal Learning ($p=0.03$ and 0.01, respectively). The slopes showed a trend for increasing Ch/Cr and better performance in healthy volunteers in Verbal Recall ($p=0.08$) and for mI/Cr in Verbal Recognition ($p=0.06$) and Hypothesis Generation ($p=0.10$). Although the differences between the PCh/Cr slopes for depressed and healthy groups in Learning, Recall, Recognition, and Hypothesis Generation

Table 2 Standardized Regression Coefficients for Metabolite Ratios and Cognitive Scales among Depressed Patients and Healthy Controls

	Controls						Depressed						Difference					
	Ch	mI	Pe	PCh	F	*p*-value	Ch	mI	Pe	PCh	F	*p*-value	Ch	mI	Pe	PCh	F	*p*-value	Model R^2
Domain																			
Learning	**0.64***	**0.83***	−0.19	**−0.85***	3.70	**0.02**	−0.28	−0.41	−0.16	−0.16	1.25	0.33	**−0.92***	**−1.24***	0.03	0.69	3.74	**0.02**	0.54
Recall	**0.63***	0.48	0.12	−0.36	1.58	0.22	−0.19	−0.27	−0.12	−0.21	0.058	0.68	−0.83	−0.76	−0.24	0.16	1.52	0.24	0.34
Recognition	**0.62***	**0.93***	0.12	−0.67	3.43	**0.03**	0.07	0.09	−0.66	−0.01	1.11	0.38	−0.55	−0.85	−0.78	0.68	1.66	0.21	0.50
Executive	0.31	−0.04	0.25	0.19	0.67	0.62	0.06	−0.08	−0.23	−0.54	1.64	0.21	−0.25	−0.03	−0.48	−0.73	1.28	0.31	0.38
Hypothesis Generation	0.59*	0.53	0.01	−0.77	1.75	0.18	0.32	−0.21	0.06	−0.08	0.50	0.75	−0.27	−0.75	0.04	0.69	0.99	0.44	0.47
Processing Speed	0.25	0.09	0.56*	0.06	1.95	0.14	0.22	−0.70*	−0.26	−0.32	3.25	0.04	−0.47	−0.79	−0.81	−0.38	3.39	0.03	0.55

Difference scores represent the depressed group's coefficients minus the control group's coefficients, or an interaction. Education served as the covariate. Significance is represented by *$p > 0.05$ with df = 10, 17 for all regression equations. Owing to the small sample size and multiple tests, the significant results that occur in a clear pattern are bolded and the ones that do not appear in a clear pattern are underlined.

were modest and nonsignificant, they were all positive. Thus, the slopes for controls were more negative than were the slopes for depressed patients in the PCh and cognition associations, indicating a different association among controls than among patients. The results must be interpreted with caution due to the small sample size, but the results that form a pattern are considered more likely to represent real than chance findings.

CONCLUSION

To our knowledge this is the first study to examine the relationship between cognition and the individual metabolites of Ch/Cr, Pe/Cr, PCh/Cr, and mI/Cr among depressed and healthy elderly volunteers. Healthy elderly subjects and patients with MDD showed different relational patterns between the metabolites measured in the left dorsolateral white matter and cognitive domains. Among healthy elderly controls, increased Ch/Cr and mI/Cr and decreased PCh/Cr were associated with better performance in verbal learning, recall, recognition, and hypothesis generation. In contrast, depressed patients showed an inconsistent pattern of relationships between metabolites and cognition. Second, there was a significant difference between patients and controls in the overall pattern of relationships between the four metabolites and cognitive performance in verbal learning and speed of processing. Third, there were no significant associations between executive function and the four metabolites or the overall model.

The difference scores between the healthy and depressed elders indicated that depressed patients did not experience an increase in cognitive function with increasing Ch/Cr and mI/Cr levels as was seen among healthy participants. Although PCh/Cr correlated negatively with cognitive function in the four domains of verbal learning, recall, recognition, and hypothesis generation for both healthy and depressed participants, the association was weaker among depressed persons than among healthy participants. The lack of benefit derived from increased Ch/Cr and mI/Cr among depressed patients suggests a loss of integrated biochemical concentrations that appear necessary for efficient cognitive function.

Ch and inositol are common precursors of phosphoglycerides, one of the three major kinds of membrane lipids (Styor, 1988). By providing *in vivo* estimates of Ch concentration, MR spectroscopy is thought to provide information on membrane turnover (Gadian, 1995), the degree of myelination (Hida *et al*, 1992), and cell density (Miller *et al*, 1996). The Ch concentration is reported to vary with the production and degradation of the Ch-containing phospholipids, which are abundant in cell membrane and in myelin (Miller *et al*, 1996; Alberts *et al*, 2002; Styor, 1988). An abnormality in membrane structure or myelination could precipitate decreased synaptic strength and efficiency, manifesting in cognitive deficits.

Changes in phospholipid turnover and gliosis are the most plausible explanations for the abnormal relationship between cognition and mI/Cr observed among depressed patients in this study (Robertson *et al*, 2001). mI is known to be abundant in glia although it is also involved in several other important cellular processes in the brain such as

cellular osmoregulation and secondary messenger trafficking (Brand *et al*, 1993; Chang *et al*, 1996; Robertson *et al*, 2001; Ross *et al*, 1996). In mild HIV brain injury, before clinical and cognitive signs of the disease are apparent but after gliosis has begun to increase, cognitive tasks require increased activated brain volume for completion, and the increase is independent of task complexity (Ernst *et al*, 2002). Thus, more activation (or mental effort) is required to maintain the same level of response performance. Post-mortem findings in patients with mood disorders demonstrated abnormalities in both neuronal and glial compartments in patients with mood disorders (Rajkowska, 2000). Abnormalities in glial function could alter the levels of neurochemicals in critical brain regions, thereby impacting on neuronal circuitry and the cognitive function dependent on that circuitry.

In conclusion, among depressed patients the brain metabolites of Ch/Cr, Pe/Cr, PCh/Cr, and mI/Cr are not associated with cognition in the same manner as seen among healthy elderly persons. While healthy elders use higher levels of Ch/Cr, mI/Cr, and Pe/Cr and a lower level of PCh/Cr advantageously, depressed patients demonstrate no clear patter of associations with the metabolites. Abnormalities in cellular membrane structure and/or gliosis could lead to alterations in frontostriatal circuitry that modulate cognitive processes.

ACKNOWLEDGEMENTS

This research was supported by NIH Grants RO1 MH 63764, RO1 MH 61567, and KO2 MH 02043 (A Kumar, PI).

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