
Biochemical Markers of Mood: A Proton Magnetic Resonance Spectroscopy Study of Normal Human Brain

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Background: *Elevated brain Cho has been shown within the basal ganglia and frontal (i.e., orbitofrontal and cingulate) cortices in patients with mood disorders utilizing Proton Magnetic Resonance Spectroscopy ($^1\text{H-MRS}$). We sought to determine the relationship between Cho and mood in a cohort of healthy young subjects.*

Methods: *Twenty-seven subjects without neurologic or psychiatric disorders were evaluated with the Positive and Negative Affect Scale and underwent $^1\text{H-MRS}$ of bilateral frontal and occipito-parietal white matter.*

Results: *We found that Cho in the left frontal lobe was inversely correlated with Positive Affect [$F(1,24) = 19.2$, $p < .001$, $r^2 = .45$].*

Conclusions: *Our results highlight the important involvement of Cho underlying the integration of affective processing within prefrontal circuitry, and may indicate increased myelin turnover in subjects with lower Positive Affect. Further efforts will be necessary to determine if high Cho is associated with increased incidence of mood disorders throughout life. Biol Psychiatry 2002;51:224–229 © 2002 Society of Biological Psychiatry*

Key Words: Depression, anxiety, affect, mood, choline, spectroscopy

Introduction

Mood regulation in humans involves a widely distributed system of interconnected brain regions including limbic, basal ganglia, and frontal-subcortical circuits (Cummings 1993; Mayberg 1999). Numerous studies show variability in frontal lobe integrity and physiology related to variation in mood (Soares and Mann 1997a, 1997b for review). Structural imaging studies have revealed increased hyperintensities (Coffey 1993) and in-

creased T_1 signal (Dolan et al 1990) within frontal white matter of depressed patients. Similarly, functional imaging studies have demonstrated “hypofrontality,” (i.e., reduced regional cerebral blood flow/glucose metabolism) across a broad range of subjects with mood disorders (Soares and Mann 1997b). While the structural and functional correlates of frontal lobe integrity to mood states have been well researched, the neurochemical correlates of these structural-functional abnormalities are poorly understood.

Proton Magnetic Resonance Spectroscopy ($^1\text{H-MRS}$) provides a quantitative measure of the neurochemical components of neuronal variation in vivo. Within the proton spectrum, signals from metabolites including *N*-acetylaspartate (NAA), creatine (Cre), and choline (Cho) are readily observed. NAA, localized predominantly in neurons and axons, has been hypothesized to be involved in lipid and protein synthesis, may serve as a neuronal osmolyte, and has been described as a marker of neuronal integrity (Barker 2001). Cre is a marker of tissue energetics, and is often used as a standard in ratio analyses of neurometabolite change (Ross 1994). Of particular interest to research of mood, the Cho resonance is comprised of numerous metabolites including glycerophosphocholine and phosphocholine (Barker et al 1994), and has been implicated in inflammation, demyelination, and membrane synthesis or repair in neurologic disorders (Ross et al 1994).

Basal ganglia Cho elevations have been well described in patients diagnosed with bipolar and major depressive disorders (Soares et al 1996; Kato 1998); however, some researchers have found lower Cho levels within the basal ganglia in treatment responsive compared to nonresponsive depression (Renshaw et al 1997), and within the hippocampus of depressed patients before treatment with electroconvulsive therapy compared to controls (Ende et al 2000). Within the frontal lobes, recent studies have revealed an increased Cho/Cre ratio within the left orbitofrontal cortex in depressed adolescents (Steingard et al 2000), and within the anterior cingulate cortex of bipolar patients (Soares et al 1999), implicating frontal lobe metabolic derangement in mood disorders. Finally, an objective measure of depression (21-item Hamilton De-

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pression Rating Scale) was significantly correlated with Cho/Cre ratios within the left anterior cingulate cortex in subjects diagnosed with bipolar disorder (Moore 2000).

It is not known whether Cho concentrations are related to variation in mood in normal individuals. Within normal brain, Cho increases with age (Pfefferbaum et al 1999), varies between individuals (Brooks et al 1999), and is anatomically heterogeneous (Barker et al 2000). Cho also varies with changes in cell density and type (Miller 1996), suggesting it may be a surrogate marker of cellularity. Among normal individuals, Tellegen (1999) has argued that mood involves two psychometrically independent factors: positive and negative affect. In the current study, we hypothesized that levels of frontal white matter Cho would underlie variations in positive and negative affect in normal human brain.

Methods and Materials

Subjects

Thirty participants were recruited from the University community and screened to exclude obvious organic or medical disease known to affect neurochemistry and mood: prior traumatic brain injury, disorders of attention, learning disability, neurologic disease, psychiatric diagnosis, use of psychoactive medications, and drug abuse. After complete description of the study to the subjects, written informed consent was obtained. This study was approved by the Institutional Review Board. Three subjects did not meet experimental criteria leaving a final sample of 27.

Magnetic Resonance Imaging and Spectroscopy

Magnetic Resonance (MR) acquisitions were obtained on a 1.5 Tesla clinical scanner (General Electric Medical Systems, Waukesha, WI). A PRESS spectroscopic pulse sequence, including water suppression, was employed (TE = 40 msec, TR = 2000 msec, 128 averages, 12.6 cm³). Three discrete voxel locations within left occipito-parietal, left frontal, and right frontal white matter, were selected to maximize white-matter and to minimize gray-matter and ventricular contribution (see Figure 1a). Spectroscopic data were analyzed using Magnetic Resonance User Interface (MRUI). Data were quantified by employing the internal water signal as a concentration reference standard and corrected for metabolites (NAA, Cre, Cho) and water T₁ and T₂ effects during echo and recycle times using literature values (Barker 1993).

Standardization of Voxel Placement

Because head tilt varies across subjects due to differences in head and body size and shape, a standardized method of voxel prescription was developed for the inferior-superior plane. On a midsagittal slice (see Figure 1b), a line (Line A) was drawn at the base of the genu and splenium of the corpus callosum (CC). A second line (Line B) was drawn perpendicular to Line A through the genu of the CC. The distance from the base of the brain to the

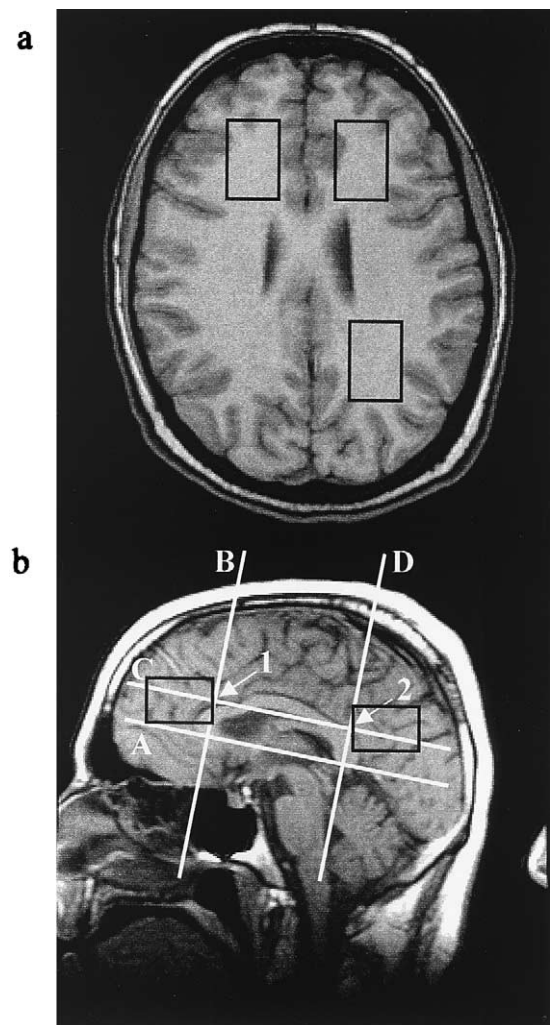


Figure 1. (a) Representative axial T₁ image and locations for the three voxels within left and right frontal white matter and left occipito-parietal white matter. (b) Graphical representation of standardization of voxel placement within frontal and parietal white matter. Representative voxels (in black) are positioned within the superior-inferior plane.

anterior aspects of the parenchyma was measured, and a point placed within the frontal lobes at 1/2 distance along line B (Point 1). This point locates the inferior-superior position of the midpoint of the frontal voxels. Next, a line (Line C) was drawn from point 1, that was parallel to Line A, and extended through the occipital lobe. Finally, a line (Line D) was drawn through the splenium of the CC perpendicular to Lines A and C. The intersection of Lines C and D (Point 2) locates the inferior-superior position of the occipital-parietal voxel. Representative voxels demonstrating the placement within frontal and occipital regions are shown (see Figure 1b).

Correction for CSF in the voxel

Since metabolite concentrations in CSF are negligible, concentrations of NAA, Cre, and Cho were corrected for brain paren-

Table 1. Descriptive Statistics and Tests of Gender Differences for the Experimental Sample, Including Age, Measures of Cho within the Three Voxel Locations, Positive Affect and Negative Affect

	Mean	SD	Min.	Max.	Skew	Kurtosis	<i>t</i> -(<i>p</i>)
Age	24.89	5.79	18	37	.649	-.652	
Females (<i>n</i> = 11)	24.55	5.50	18	36			-.25
Males (<i>n</i> = 16)	25.13	6.14	18	37			(.80)
Positive Affect	32.40	6.64	22	43	.125	-1.150	
Females (<i>n</i> = 11)	32.64	7.34	22	43			.15
Males (<i>n</i> = 16)	32.25	6.36	22	43			(.88)
Negative Affect	13.89	3.70	10	26	1.420	2.850	
Females (<i>n</i> = 11)	15.00	4.33	11	26			1.31
Males (<i>n</i> = 16)	13.12	3.12	10	19			(.20)
Cho Left Frontal	1.80	.27	1.31	2.48	.374	.239	
Females (<i>n</i> = 11)	1.75	.26	1.31	2.21			-.76
Males (<i>n</i> = 16)	1.83	.28	1.45	2.48			(.45)
Cho Right Frontal	1.71	.28	1.16	2.40	.606	.775	
Females (<i>n</i> = 11)	1.61	.22	1.16	2.08			-1.54
Males (<i>n</i> = 16)	1.78	.30	1.36	2.40			(.14)
Cho Left Occipital	1.58	.22	1.18	1.98	-.059	-.745	
Females (<i>n</i> = 11)	1.49	.21	1.18	1.83			-1.78
Males (<i>n</i> = 16)	1.64	.20	1.25	1.98			(.09)

Cho, choline.

chyma fraction in each voxel. T_1 weighted images were segmented using a k-means clustering algorithm that classifies spatial information into clusters based on the intensity of the image pixels as described previously (Petropoulos 1999). Each output cluster represents a different tissue type: white matter, gray matter, cerebrospinal fluid (CSF), and partial volume gray/CSF. The fraction of tissue in the voxel was then determined by subtracting the number of pixels corresponding to CSF from the total number of pixels comprising the voxel and dividing by the number of pixels. The pixels corresponding to partial volume gray/CSF were assigned evenly between CSF and brain tissue. Thus, MRS values for NAA, Cre, and Cho were corrected within each voxel (Mean tissue content across voxels = .96, SD = .02).

Mood Measure

The Positive Affect Negative Affect Scale (PANAS) was used to assess two relatively orthogonal dimensions of mood (Watson 1988). Positive affect refers to "the extent to which a person feels enthusiastic, active and alert." Consequently, low Positive Affect is characterized by sadness and lethargy, while Negative Affect is more a measure of psychological distress (e.g., anger, disgust, fear). Tellegen (1999) has suggested that low Positive Affect is a distinguishing feature of depression, while high Negative Affect is characteristic of anxiety. The PANAS is brief, easy to administer, internally consistent, and stable over a 2-month time period. While the PANAS is not a measure of mood dysfunction per se, it does tap the fundamental attributes of normal and pathologic mood states, and correlates well with other measures of mood dysfunction including the Beck Depression Inventory, and the Symptom Checklist-90 (Watson et al 1988).

Statistical Methods

Spearman correlation coefficients were used to assess the initial relationships between age, affect (Positive Affect, Negative Affect) and major metabolites (NAA, Cre, Cho) within the three voxel locations. Differences between men and women across major experimental variables were assessed using *t* tests. Linear regression models were used to assess potential predictors (NAA, Cre, Cho within the three voxel locations) of positive and negative affect, the dependent variables. Statistical analysis was conducted in SPSS for Macintosh (SPSS Inc. 1995).

Results

Complete spectroscopy and PANAS measures were obtained from 27 experimental subjects (11 female, 16 male; mean age 25 ± 5.8 years). Women did not differ significantly from men in terms of age, affect (Positive or Negative), or Cho across the three voxel locations (see Table 1).

Mean mood scores (Positive Affect = 32.40 ± 6.64 ; Negative Affect = 13.89 ± 3.70) were well within the range of normal control values (Watson 1988); however, scatter plots revealed that Negative Affect was strongly skewed to the low end of the scale values, while Positive Affect was normally distributed. The correlation between Positive Affect and Negative Affect was low ($r = .18$, $p = .37$).

Intraindividual correlations among the Cho variables within the three voxel locations ranged from $r = .78$ to $r = .71$ (each $p < .001$). Age was significantly correlated with Cho in the left frontal ($r = .42$, $p < .03$), right frontal ($r =$

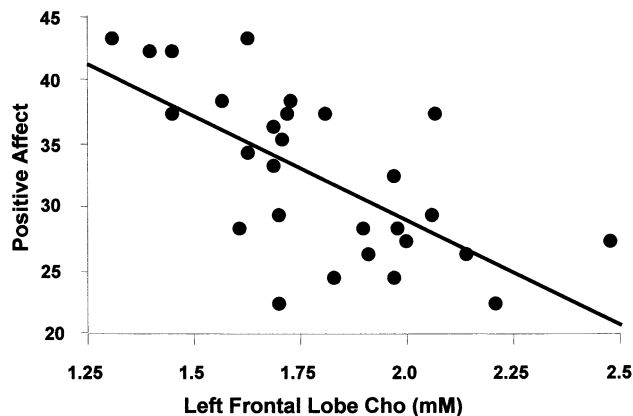


Figure 2. Scatterplot of Choline within the left frontal white matter and Positive Affect across the entire sample. Cho, choline.

.48, $p < .02$), and left occipital ($r = .47$, $p < .02$) voxels. Consequently, age was partialled out in initial correlation analyses.

In our initial analysis, we found that Cho was inversely correlated with Positive Affect in the left frontal lobe ($r = -.61$, $p = .001$); however, correlations between Cho and right frontal ($r = -0.35$, $p = .08$) or left occipito-parietal voxels ($r = -.24$, $p = .25$) failed to reach significance. Neither NAA nor Cre in any voxel location were significantly related to Positive or Negative Affect. Similarly, Negative Affect was not related to Cho in any voxel location. Finally, tissue content within each of the three voxels was not significantly related to Positive or Negative affect or Cho. In women, the partial correlation of Cho with Positive Affect was $r = -.82$ ($p < .004$) versus $r = -.47$ ($p < .08$) in men. These correlations did not differ significantly as might be expected given small sample sizes.

To assess the regional contribution of neurometabolism to affect, we regressed Cho measures from the three voxel locations, and age, against Positive Affect, the dependent variable. A model including only left frontal Cho best predicted Positive Affect [$F(1,24) = 19.2$, $p < .001$, $r^2 = .45$]. Figure 2 demonstrates the linear relationship between left frontal lobe Cho and Positive Affect for the experimental sample. Figure 3 demonstrates example spectra from the extremes of the Cho distribution.

Discussion

To our knowledge, these results provide the first evidence that individual variation in neurochemical concentrations detected by ^1H -MRS are related to mood in normal individuals; however, our results must be interpreted with caution for several reasons. Our sample size was small and lacking sufficient power to evaluate possible gender differences in the magnitude of the relationship between Cho

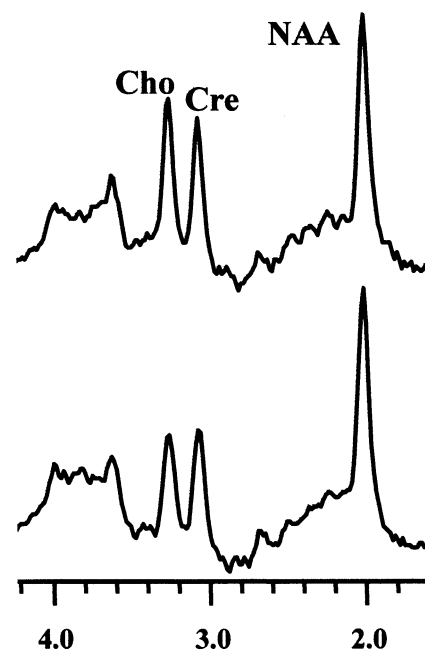


Figure 3. Example spectra of two experimental subjects displaying relatively high (upper spectrum) and low (lower spectrum) levels of Cho. Units are in parts per million (ppm). Cho, choline; Cre, creatine; NAA, *N*-acetylaspartate.

and Positive Affect. We examined Cho in only three discrete voxels and, although lower Cho in each voxel predicted greater levels of Positive Affect, the effect was only significant in the left frontal voxel. Magnetic Resonance Spectroscopic Imaging with a larger sample would provide a clearer understanding of the importance of regional variations in neurochemical levels to the experience of normal affect.

No statistically significant relationship was observed between Negative Affect and Cho. While this negative result may reflect limited variability in Negative Affect in our normal sample, it is also possible that Cho has a differential impact on the neural systems related to depression (i.e., low Positive Affect) and anxiety (i.e., high Negative Affect). Indeed, Depue (1994) has emphasized that positive affect may reflect activity in a fronto-limbic dopaminergic circuit, while Gray (1985) has suggested that activity in septo-hippocampal gabaminergic systems relate to anxiety. Other researchers have described relatively discrete corticolimbic pathways associated with sadness (i.e., dorsal cortical deactivation) and anxiety (i.e., ventral cortical deactivation) in normal healthy women (Liotti et al 2000).

Changes in nuclear magnetic relaxation processes may account for the apparent differences observed in Cho concentrations within our experimental sample. If present, such a finding would suggest a different interpretation of our results. In particular, Dolan et al (1990) report small,

but statistically significant, elevations in T_1 (spin-lattice relaxation) in frontal brain in depressed patients. Although our spectroscopic data were acquired under somewhat saturated conditions ($TR = 2000$ msec), two lines of evidence suggest that this would not account for our observations. First, since an elevation in T_1 seen on MRI most likely represents the intracellular milieu, we can assume that such an effect would impact all metabolites; however, our data show that Cho is the only metabolite examined to reveal any significant correlation with mood, suggesting that we are observing a Cho-specific mechanism. Second, under our acquisition conditions, an elevation of T_1 would actually reduce the apparent concentration of Cho rather than increase it as we have seen in subjects with low Positive Affect. Thus, while potential relaxation effects should be carefully considered, they do not appear to underlie our observations.

Issues deserving further study include the specific contribution of age and gender to the observed relationship between Cho and Positive Affect. For example, even though our sample was relatively young, increasing age predicted higher Cho in each voxel examined. Future studies will need to examine a broader age range to evaluate the possibility that Cho variability is a vulnerability or risk factor associated with mood dysfunction across the life span. Similarly, we found higher correlation coefficients between Positive Affect and Cho in women than in men, although these differences did not reach statistical significance. It is possible that phase of menstrual cycle, known to affect hormonal status in women, may contribute to body water metabolism and/or Cho resonance within female subjects. This takes on further importance considering we used voxel water to normalize metabolic concentrations. While we did not control for menstrual cycle in our study, recent research has suggested that the Cho/Cr ratio is stable within parietal and frontal cortices across follicular and luteal phases of the menstrual cycle in normal women (Rasgon et al 2001).

With the acquisition technology used, it is difficult to distinguish which of the numerous metabolites underlying the Cho resonance (e.g., phosphorylcholine, glycerophosphorylcholine, phosphatidylcholine, sphingomyelin) may best explain the observed relationship to mood functioning within our experimental sample. We have speculated that higher Cho may reflect greater myelin turnover (Jung et al 1999), as the Cho peak in 1H -MRS includes both precursors and breakdown products of myelin. Preliminary support for this notion is found in research linking Cho to membrane phospholipid metabolism (Miller 1991), as well as developmental changes in the constituents of the Cho resonance (e.g., phosphorylcholine, phosphorylethanolamine) linked to developmental myelination (Bluml et al 1999). Our results would suggest that study of myelinated

axons within the frontal lobes of subjects across the spectrum of mood functioning might yield critical insights into metabolic abnormalities associated with mood.

The importance of Cho in predicting mood is consistent with an emerging clinical 1H -MRS literature (Steingard 2000; Kato 1998), as well as the observation that administration of choline may produce depression (Ananth and Ghadirian 1980). Similarly, administration of lithium to patients with bipolar disorder yields reduction of Cho levels within the frontal lobe (Moore 1999), implicating Cho as a putative marker of symptom reduction in mood disorders. Numerous hypothetical roles have been ascribed to the Cho resonance, including (1) that Cho is a precursor to acetylcholine, critical to cholinergic-adrenergic equilibrium (Janowsky et al 1972); (2) that a component of Cho (i.e., phosphatidylcholine) is associated with intracellular signal transduction (Exton 1994); (3) that changes in Cho may be related to changes in cerebral oxidative metabolism (Duc et al 1997); and (4) that changes in Cho may reflect endocrine status (e.g., hypothyroidism) associated with mood disorders (Gupta et al 1995). Taken together with these hypotheses, our results highlight the important involvement of Cho underlying the integration of affective processing within prefrontal circuitry (Mesulam 1986; Sackeim et al 1990).

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