

# Chemical Heterogeneity of the Living Human Brain: A Proton MR Spectroscopy Study on the Effects of Sex, Age, and Brain Region

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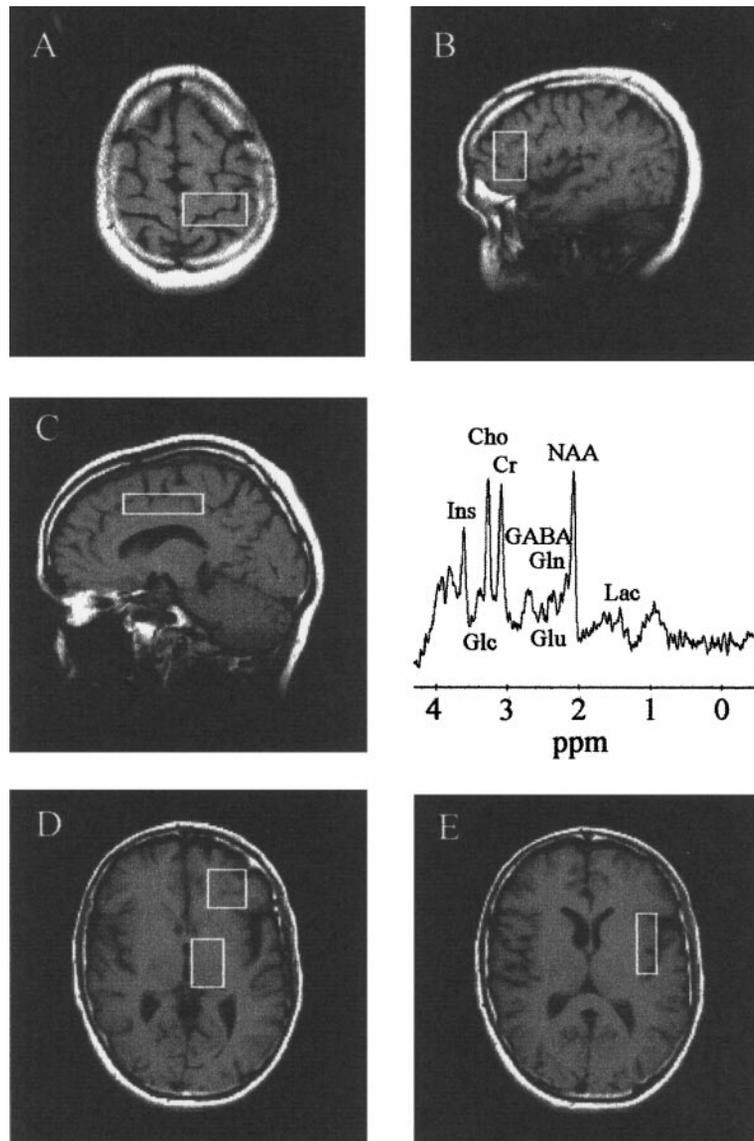
**Brain chemistry was compared between 19 male and female normal volunteers in the age group 19–31 years, across six brain regions and nine metabolites using *in vivo* proton magnetic resonance spectroscopy. The relative concentrations of *N*-acetyl aspartate, choline, glutamate, glutamine, GABA, inositol, glucose, and lactate were measured relative to creatine within 8-cm<sup>3</sup> brain voxels. These measurements were performed in six brain regions: thalamus and cingulate, insula, sensorimotor, dorsolateral prefrontal, and orbital frontal cortices in the left hemisphere. Total metabolite concentration was highest in prefrontal regions (28% higher in orbital frontal cortex and 18.7% higher in dorsolateral prefrontal cortex compared with insula and thalamus,  $P < 10^{-7}$ ). Subjects 25–31 years of age demonstrated a significant increase in total metabolite concentration in the orbital frontal cortex (35%,  $P < 10^{-7}$ ) and sensorimotor cortex (16.7%,  $P < 10^{-5}$ ) compared to those 19–20 years of age. These two brain regions also showed gender dependence, with women demonstrating increased metabolite concentrations compared to men (9% increase in sensorimotor cortex,  $P < 0.002$ , and 2.1% in orbital frontal cortex). Most other brain regions showed no gender- or age-dependent differences. The results indicate that the living human brain is chemically heterogeneous. The chemical heterogeneity is sex and age dependent and specific for brain region.** © 2000 Academic Press

## INTRODUCTION

Until recently, it has been technically impossible to identify and quantify the specific cellular and molecular mechanisms of neuronal activity using *in vivo*

brain-imaging technology. A number of advances in magnetic resonance imaging have made the latter possible. As a result magnetic resonance spectroscopy (MRS) has become a useful and increasingly powerful technique for studying chemistry of the living brain, which can be viewed as a noninvasive and nondestructive biopsy of living tissue (Lock *et al.*, 1990; Stanley *et al.*, 1995). *In vivo* MRS is similar to MRI, except that it uses the slight differences in resonance frequency between different chemical groups to measure the regional concentrations of brain chemicals (Shulman and Rothman, 1998). A proton (<sup>1</sup>H) MRS spectrum contains information on multiple metabolites and provides a window into the energy metabolism of living brain cells. *In vivo* <sup>1</sup>H MRS studies of the brain are capable of quantifying steady-state metabolic levels of neurotransmitters such as glutamate (Glu), *N*-acetyl aspartate (NAA), and  $\gamma$ -aminobutyric acid (GABA), which are predominantly localized within neurons and may therefore reflect neuronal functional–synaptic properties (Miller, 1991; Stanley *et al.*, 1995; Castillo *et al.*, 1998; Shulman and Rothman, 1998; Magistretti *et al.*, 1999). <sup>1</sup>H MRS also measures other chemical messengers and small molecules such as choline (Cho), glutamine (Gln), myo- and scyllo-inositol (Ins), glucose (Glc), lactate (Lac), and creatine and phosphocreatine complex (Cr), which are involved in the metabolic pathway of the tricarboxylic acid (TCA) cycle, neuronal and astrocytic neurotransmitter cycling, and membrane turnover (Miller, 1991; Michaelis *et al.*, 1993; Stanley *et al.*, 1995; Gruetter *et al.*, 1996; Shulman and Rothman, 1998; Sibson *et al.*, 1998; Magistretti *et al.*, 1999). <sup>1</sup>H MRS has been used extensively to examine brain pathology, even though the distribution of metabolites in the normal brain has been studied to a limited extent. For example, abnormal NAA concentrations have been observed in epilepsy, dementias, stroke, hypoxia, multiple sclerosis, and leukoencephalopathies (Salibi and Brown, 1998). Choline (i.e., mostly the choline-containing compound glycerylphosphorylcholine) is thought of as a product of sphingomyelin (a principal structural lipid of the myelin sheath) and found to be

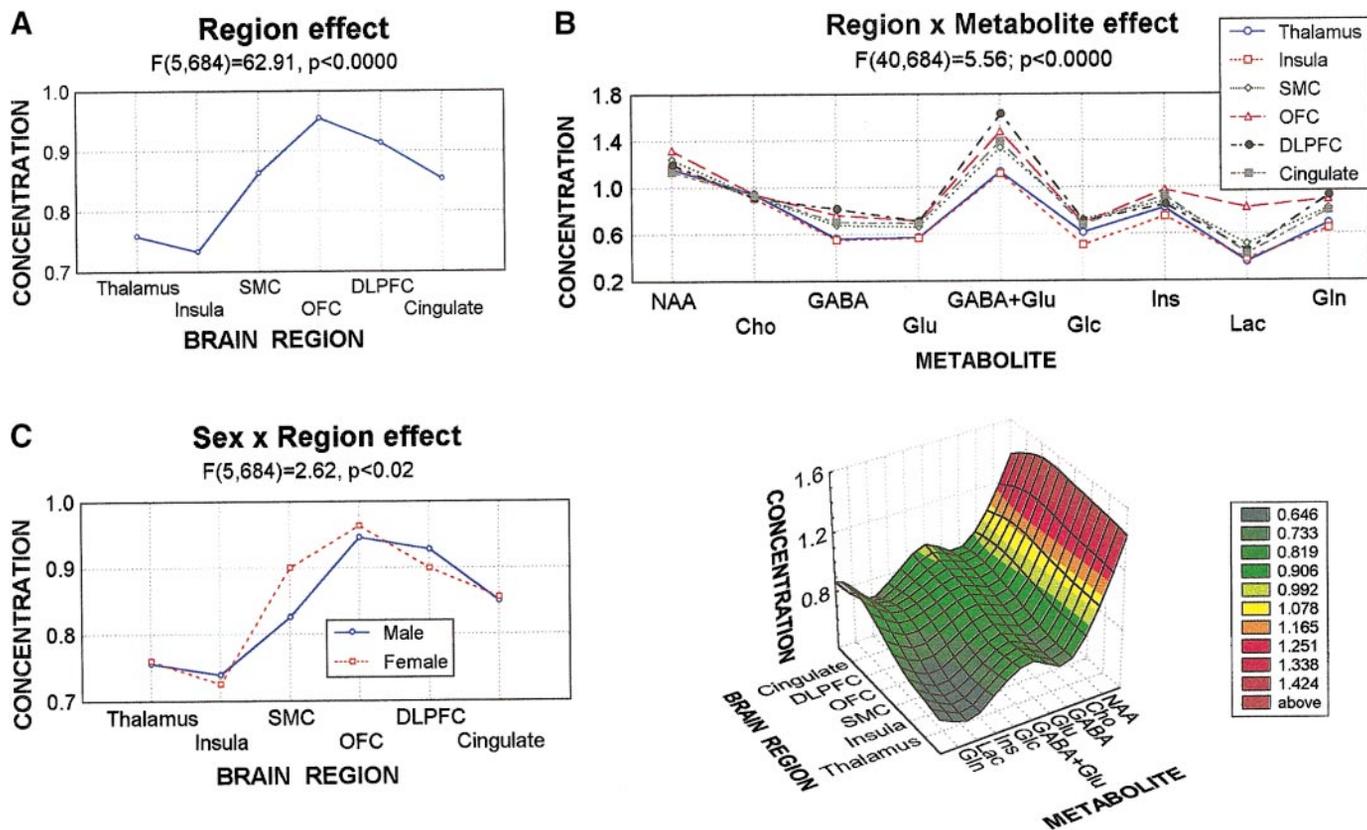
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**FIG. 1.** Selection of volumes of interest for *in vivo* three-dimensional proton MRS brain examination. T1-weighted spin echo high-resolution images showing the locations of six voxels in the left hemisphere of a normal subject. Axial planes showing the voxel size and midslice positioning of region of interest (ROI) for SMC (A), thalamus (lower box) and OFC (D), and insula (E). Sagittal planes showing the voxel size and midslice positioning of ROI for DLPFC (B) and cingulate (C). Typical *in vivo* proton MRS spectra (C) obtained from one of the ROI (cingulate) showing localization of three major peaks for NAA (2.02 ppm), Cr (3 ppm), and Cho (3.2 ppm) and several small peaks for Glu (2.35 ppm), Gln (2.15 ppm), GABA (2.25 ppm), Ins (3.60 ppm), Glc (3.43 ppm), and Lac (1.3 ppm). Chemical shifts are indicated in parts per million (ppm). Localized proton spectra were acquired using a simulated-echo acquisition mode sequence (probe-s PSD, TR = 1500 ms, TE = 30 ms), 8 cm<sup>3</sup> voxel size.

abnormal in Alzheimer's disease, chronic hypoxia, post-liver transplant, epilepsy, and hepatic encephalopathy (Salibi and Brown, 1998). <sup>1</sup>H MRS has also opened a new avenue to the study of brain chemistry in neuropsychiatric disorders such as schizophrenia and bipolar disorder. A decrease in the NAA and Glu level was observed in the left dorsolateral prefrontal cortex of never-treated schizophrenic patients (Stanley *et al.*, 1995), and decrease of NAA concentration was ob-

served in the right hippocampus/amygdala complex of patients with schizophrenia receiving antipsychotic medications (Nasrallah *et al.*, 1995). One <sup>1</sup>H MRS study of patients with bipolar disorder who were being treated with lithium found elevated choline/phosphocreatine concentration in basal ganglia, however, no significant differences in relative choline concentration were found in another similarly designed study (Keshavan *et al.*, 1995).



**FIG. 2.** Effects of Region and the Region  $\times$  Metabolite and Sex  $\times$  Region interactions on the total metabolite concentration in the living human brain. (A) Main effect of brain Region on metabolite concentration. The highest level of total metabolite concentration is in OFC and DLPFC and the lowest level in the insula and thalamus [ $F(5, 684) = 62.91, P < 0.0000$ ]. (B) Main effect of Region  $\times$  Metabolite interaction on metabolite concentration in 2D and 3D plot (see below). The highest concentrations of all metabolites were in prefrontal regions [ $F(40, 684) = 5.56, P < 0.0000$ ]. (C) Main effect of Sex  $\times$  Region interaction on metabolite concentration. Increased levels of the total metabolite concentration are in SMC of females compared to males [ $F(5, 684) = 2.62, P < 0.02$ ].

Only a few *in vivo*  $^1\text{H}$  MRS studies have examined age-dependent changes of the human brain chemistry (van der Knaap *et al.*, 1990; Peden *et al.*, 1990; Bruhn *et al.*, 1992; Kreis *et al.*, 1993; Toft *et al.*, 1994a, 1994b; Cohen *et al.*, 1995). The majority of these studies consistently showed an increase in relative concentrations of NAA from neonates to 16 years of age and were limited to a few brain regions (occipital lobe or paraventricular white/gray matter). Decreased uptake of choline in the basal ganglia of older adults (ages 60–85) was shown by Cohen *et al.* (1995). The current study is the first in which brain neurochemistry is compared between male and female normal volunteers in early adulthood (ages 19–31). Specifically we studied sex- and age-dependent changes in the concentrations of NAA, GABA, Glu, GABA+Glu, Gln, Cho, Ins, Glc, and Lac relative to Cr (internal standard), in six brain regions including thalamus, insula, sensorimotor cortex (SMC), orbital frontal cortex (OFC), dorsolateral prefrontal cortex (DLPFC), and cingulate, using *in vivo* localized three-dimensional (3D)  $^1\text{H}$  MRS methodology (Fig. 1).

## MATERIALS AND METHODS

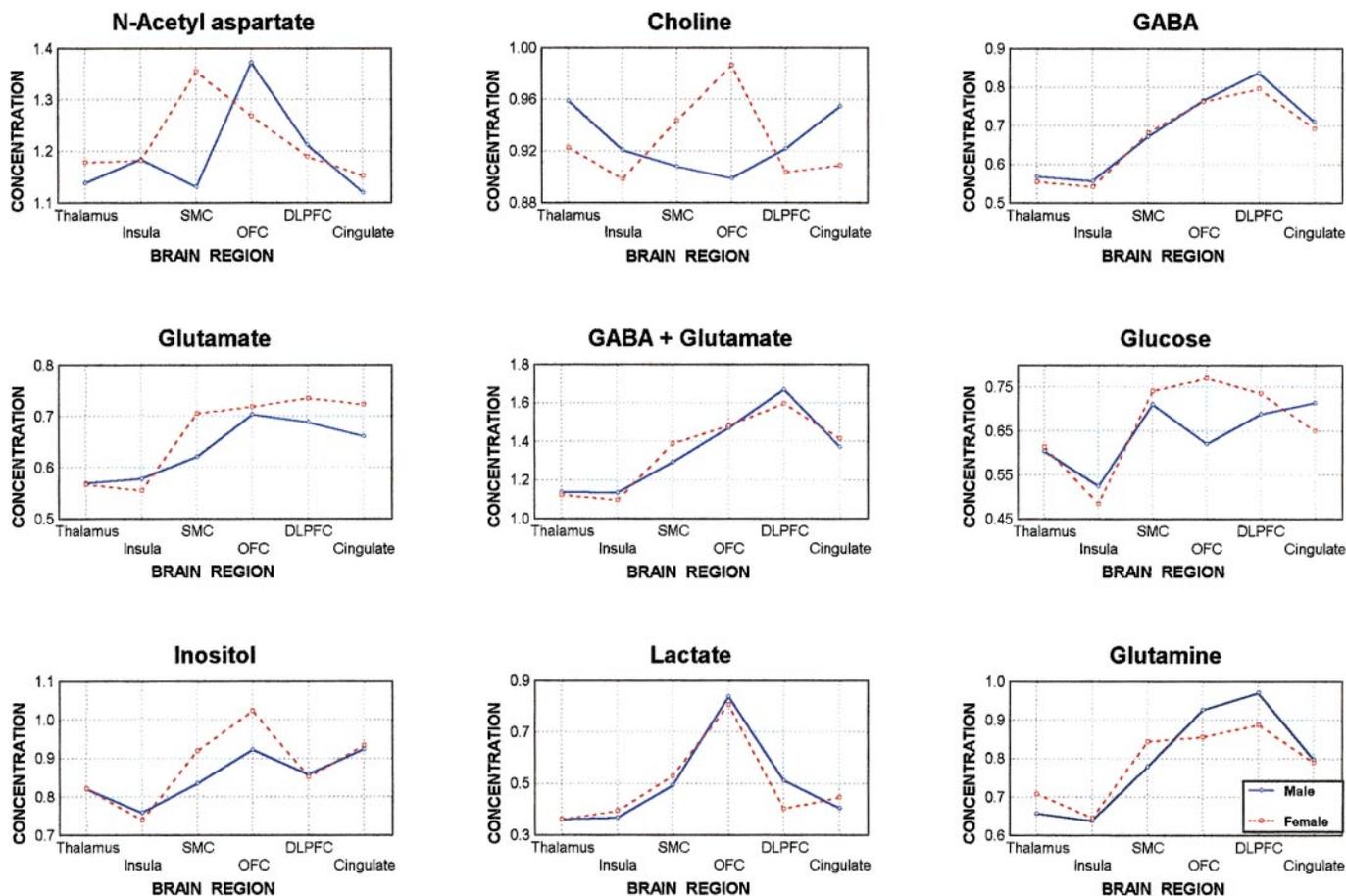
### Subjects

Nineteen normal right-handed volunteers (11 men and 8 women) participated in the proton MRS study. All subjects were students within our institution, i.e., medical students, graduate students, and residents. The general purpose and the procedures were explained to the subjects. All subjects were in the 19–31 age range and signed a consent form. The Institutional Review Board approved all procedures in this study.

### Localized *in Vivo* 3D $^1\text{H}$ MRS Brain Examination Studies

#### Patient Positioning

During each imaging session the subject was positioned on the scanner bed, and the whole head gradient coil was positioned over the head, oriented parallel to the long axis of the magnet. The subject's head was immobilized using a vacuum beanbag (Olympic Vac-Pac; Olympic Medical) shaped to the individual's head.



**FIG. 3.** Metabolite-specific sex differences for concentration in six regions of the human brain. Sex differences in relative concentrations of *N*-acetyl aspartate, choline, GABA, glutamate, GABA plus glutamate, glucose, myo- and scyllo-inositol complex (inositol), lactate, and glutamine are shown. There is increased concentration of *N*-acetyl aspartate in the SMC of females and increased glucose level in OFC of females compared with the same regional metabolites in males.

### Global Shimming

Global shimming is a procedure for optimizing the magnetic field homogeneity over the entire brain volume. Automated shimming has been used as a part of the MRS software package SPECTRO (General Electric). These procedures are fast and ensure good quality images and spectra.

### Acquisition of Magnetic Resonance Images for Localization

All MRI and MRS experiments were performed on a 1.5-T General Electric (Signa) clinical imaging instrument equipped with an Instascan resonant gradient accessory from Advanced NMR Systems, Inc., thus allowing the acquisition of both conventional and echo-planar images. High-resolution sagittal and axial views were used for the selection of a volume of interest. T1-weighted multislice spin echo scout images (TR = 500 ms; TE = 12 ms; 2NEX; 256 × 256 matrix; FOV 24 × 24 cm) of the entire brain were obtained with

6.0-mm slice thickness and a 0.5-mm gap between slices, and images were taken in 20 slice locations.

### Selection of Volume of Interest

Localized 3D <sup>1</sup>H MRS was then performed in four axial (thalamus, insula, OFC, and SMC) and in two sagittal (cingulate and DLPFC) locations in the left hemisphere (dominant) of right-handed normal volunteers. The right hemisphere was not studied because of time limitations. The scan time for each region of interest (ROI) was 6.5 min with a total scanning time for all regions of about 1 h. Positioning of each 8-cm<sup>3</sup> voxel was performed by an experienced neuroanatomist and adjusted to the individual brain's sulcal topography. To avoid partial volume effects, minimizing the mixture of gray and white matter, we used the same voxel size for each analyzed ROI (Figs. 1A–1E): 3.0 × 1.8 × 1.5 cm for thalamus, 4.0 × 2.0 × 1.0 cm for insula, 1.7 × 3.0 × 1.6 cm for DLPFC, 2.0 × 1.1 × 3.5 cm for SMC, 5.0 ×

TABLE 1

The Relative Concentrations of *N*-Acetyl Aspartate, Choline,  $\gamma$ -Aminobutyric Acid, Glutamate, Glutamine, Glucose, Inositol, and Lactate (Mean  $\pm$  SD), in Relation to the Concentration of Creatine (Internal Standard), across Six Brain Regions in Normal Subjects, Age Group 19–31 Years ( $n = 19$ )

Metabolites	Brain regions						
	Cingulate	DLPFC	OFC	SMC	Insula	Thalamus	All regions
<i>N</i> -Acetyl aspartate	1.13 $\pm$ 0.11	1.20 $\pm$ 0.10	1.32 $\pm$ 0.20	1.22 $\pm$ 0.16	1.18 $\pm$ 0.06	1.16 $\pm$ 0.08	1.20 $\pm$ 0.13
Choline	0.93 $\pm$ 0.10	0.91 $\pm$ 0.05	0.94 $\pm$ 0.13	0.92 $\pm$ 0.11	0.91 $\pm$ 0.05	0.94 $\pm$ 0.05	0.93 $\pm$ 0.08
$\gamma$ -Aminobutyric acid	0.70 $\pm$ 0.08	0.82 $\pm$ 0.07	0.76 $\pm$ 0.19	0.67 $\pm$ 0.12	0.55 $\pm$ 0.05	0.56 $\pm$ 0.06	0.67 $\pm$ 0.14
Glutamate	0.69 $\pm$ 0.08	0.71 $\pm$ 0.05	0.71 $\pm$ 0.15	0.65 $\pm$ 0.14	0.57 $\pm$ 0.05	0.57 $\pm$ 0.04	0.64 $\pm$ 0.11
$\gamma$ -Aminobutyric acid and glutamate	1.39 $\pm$ 0.14	1.63 $\pm$ 0.13	1.47 $\pm$ 0.32	1.32 $\pm$ 0.26	1.12 $\pm$ 0.10	1.13 $\pm$ 0.08	1.33 $\pm$ 0.26
Glutamine	0.79 $\pm$ 0.08	0.93 $\pm$ 0.10	0.89 $\pm$ 0.15	0.80 $\pm$ 0.20	0.64 $\pm$ 0.07	0.68 $\pm$ 0.06	0.78 $\pm$ 0.15
Glucose	0.68 $\pm$ 0.10	0.71 $\pm$ 0.05	0.69 $\pm$ 0.14	0.72 $\pm$ 0.22	0.51 $\pm$ 0.05	0.61 $\pm$ 0.05	0.64 $\pm$ 0.13
Inositol	0.93 $\pm$ 0.19	0.85 $\pm$ 0.07	0.97 $\pm$ 0.29	0.87 $\pm$ 0.11	0.75 $\pm$ 0.06	0.82 $\pm$ 0.04	0.85 $\pm$ 0.15
Lactate	0.42 $\pm$ 0.07	0.46 $\pm$ 0.12	0.82 $\pm$ 0.39	0.51 $\pm$ 0.13	0.38 $\pm$ 0.06	0.36 $\pm$ 0.04	0.47 $\pm$ 0.21
All metabolites	0.85 $\pm$ 0.29	0.91 $\pm$ 0.33	0.95 $\pm$ 0.34	0.85 $\pm$ 0.30	0.73 $\pm$ 0.27	0.76 $\pm$ 0.26	0.84 $\pm$ 0.31

1.6  $\times$  1.0 cm for cingulate, and 2.0  $\times$  2.0  $\times$  2.0 cm for OFC.

#### MRS Data Collection

Proton-localized spectra were collected using a simulated-echo acquisition mode (STEAM) sequence (probe-s PSD, TR = 1500 ms, TE = 30 ms). All spectra were collected from identical-sized voxels, which were specific for each ROI.

#### MRS Data Processing

All spectra were transformed into a standardized scale using the Scion Image analysis package (1998, see the Web site <http://www.scioncorp.com>). Proton spectra were analyzed by measuring heights at specified peaks, with the spectroscopist (I.D.G.) blinded to both the location and the subject. The relative concentrations of NAA, Cho, Glu, Gln, GABA, Ins, Glc, and Lac were measured relative to concentration of Cr, which is commonly used as an internal standard. These relative metabolite concentrations are presented in the graphics and discussed in this paper. Figure 1C shows typical proton MRS spectra of the normal human brain. The long TE spectra are usually characterized by three major peaks: NAA at 2.02 ppm, Cr at 3.0 ppm, and Cho at 3.2 ppm. NAA is the dominant peak in normal adult brain spectra. The other observable metabolites measured in our study were Glu, 2.35 ppm; Gln, 2.15 ppm; GABA, 2.25 ppm; Ins, 3.60 ppm; Glc, 3.43 ppm; and Lac, 1.3 ppm (Fig. 1C). These smaller peaks were contaminated by signals from other metabolites and proteins, although the prominent signal was from the chemicals with which we identify these peaks (Salibi and Brown, 1998).

#### Statistical Analysis

Differences in sex, age, and brain region effects on chemical concentration were analyzed with analysis of

variance (ANOVA), using the general linear model (Statistica, Tulsa, OK). The outcome variable was taken to be the metabolite concentration. Age and sex effects were analyzed separately. In these three-way ANOVAs, sex, age, brain region, metabolite, and subject were used as explanatory variables.

## RESULTS

Table 1 summarizes the original data for the relative metabolite concentration in six brain regions of the studied population. First we performed a three-way ANOVA to test whether  $^1\text{H}$  MRS can detect differences in brain metabolite concentrations across regions and between male and female subjects. A 2 (Sex)  $\times$  6 (Region)  $\times$  9 (Metabolite)-measures ANOVA revealed a significant multivariate main effects for Region [ $F(5, 684) = 62.91, P < 10^{-7}$ ], Metabolite [ $F(8, 684) = 395.23, P < 10^{-7}$ ], the Sex  $\times$  Region interaction [ $F(5, 684) = 2.62, P < 0.02$ ], and the Region  $\times$  Metabolite interaction [ $F(40, 684) = 5.56, P < 10^{-7}$ ].

The strongest significant multivariate main effects were observed for Region, Metabolite, and the Region  $\times$  Metabolite interaction (all  $P < 10^{-7}$ ). The total metabolite concentration was found to be 28% higher in OFC and 18.7% higher in DLPFC compared with insula and thalamus ( $P < 10^{-7}$ ; post hoc Sheffe test) in which the total metabolite concentration was the lowest (Fig. 2A). The concentration of metabolites in SMC remained at the level comparable with the cingulate, and both of these regions were positioned in the middle of the total brain metabolite level showing only 16.7 and 12.2% increase in concentration compared with the insula and thalamus ( $P < 10^{-7}$ ). Over all regions, metabolite concentrations were significantly different for most chemicals ( $P < 10^{-7}$ ), except between GABA vs Glc or Glu, and Glu vs Glc. Although each metabolite behaved differently in each specific brain region, the overall concentration shifts for all nine metabolites

were the highest in the prefrontal regions (Fig. 2B, all  $P < 10^{-7}$ ).

The weakest significant multivariate main effects were observed for the Sex  $\times$  Region interaction ( $P < 0.02$ ). Sex-related differences were seen over all metabolites in the SMC ( $P < 0.002$ ), with a 9% increase in females. Women also demonstrated an increase of total metabolite concentration in OFC by 2.1%, which was not significant in Sheffe analysis ( $P > 0.05$ ), and showed a trend toward a decrease of total metabolite concentration in DLPFC by 3.3% ( $P < 0.08$ ) compared with men (Fig. 2C). These regional differences may be related to sexually dimorphic patterns in brain development.

Regional analysis of distribution and an impact of each chemical on the total metabolite differences between men and women revealed metabolite-specific sex differences (Fig. 3). In the SMC of females, increased metabolite levels were detected for NAA by 20% ( $P < 0.002$ ). Increases in SMC metabolites in females were also seen for Glu by 13.4%, Ins by 10.8%, Gln by 8.3%, and GABA+Glu by 7.7%. In the OFC of women increased metabolite levels were seen for Glc by 24.2% ( $P < 0.05$ ), Ins by 11.2%, and Cho by 9.8%, while decreased metabolite levels were observed for Gln by 8.8% and for NAA by 8.4%. The concentration of metabolites detected in DLPFC of women was decreased for Lac by 27% ( $P < 0.09$ ), Gln by 9.2%, and GABA by 5% and increased for Glc by 7% and Glu by 6.5%. These changes are relative to those seen in males in the corresponding brain regions and are statistically significant only where indicated. Thus, sexually dimorphic patterns in brain development may reflect the processes of neurochemical maturation of sensorimotor and prefrontal regions which may proceed at different rates and directions in men and women.

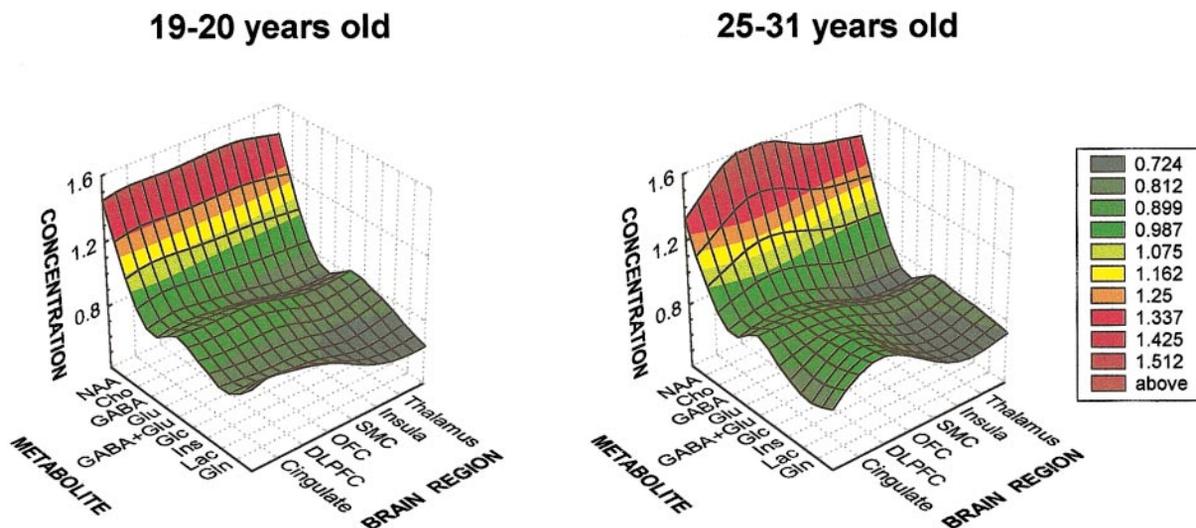
Then, we used a three-way ANOVA to test whether  $^1\text{H}$  MRS can detect differences in brain metabolite concentration between age groups across regions. A 5 (Age)  $\times$  6 (Region)  $\times$  9 (Metabolite)-measures ANOVA revealed significant multivariate main effects for Age [ $F(4, 684) = 7.48, P < 0.000009$ ], Region [ $F(5, 684) = 55.21, P < 10^{-7}$ ], Metabolite [ $F(8, 684) = 365.80, P < 10^{-7}$ ], the Age  $\times$  Region interaction [ $F(20, 684) = 13.27, P < 10^{-7}$ ], and for the Region  $\times$  Metabolite interaction [ $F(40, 684) = 4.80, P < 10^{-7}$ ]. Significant age-related differences in metabolite concentration existed in the effect on regional brain metabolism in the OFC ( $P < 10^{-7}$ ) and SMC ( $P < 10^{-5}$ ) (Fig. 4). Subjects 25–31 years of age demonstrated a significant increase in total metabolite concentration in the OFC (35%) and SMC (16.7%) compared with the 19- to 20-year-old group. These differences may reflect processes of learning and cognitive maturation during the second and third decades of life.

Analysis of variance revealed a significant main effect for the Age  $\times$  Region  $\times$  Metabolite interaction

[ $F(160, 684) = 1.30, P < 0.02$ ]. The post hoc Sheffe test demonstrated age dependency for Lac ( $P < 0.00002$ ), GABA+Glu ( $P < 0.02$ ), and GABA ( $P < 0.09$ ) over all regions (Fig. 5). The strongest regional age effects were seen in the OFC. Specific chemical concentration shifts were observed in the Age  $\times$  Region interaction for NAA ( $P < 0.0001$ ), GABA ( $P < 0.00003$ ), Glu ( $P < 0.03$ ), GABA+Glu ( $P < 10^{-7}$ ), Gln ( $P < 0.00005$ ), Lac ( $P < 10^{-7}$ ), and Ins ( $P < 0.00005$ ), indicating that their concentrations increased with advancing age in OFC. SMC also showed age-dependent increases in GABA+Glu ( $P < 0.02$ ). These changes possibly reflect activities in neuronal and astrocytic neurotransmitter and TCA cycling which can be age- and region-specific and relate to learning and memory processes during this stage of life.

## DISCUSSION

These results indicate that the living human brain is chemically heterogeneous in regard to sex, age, and brain region. These sex- and age-related chemical heterogeneities are region specific and are seen mainly in the OFC and SMC. Sex-related differences due to increased levels of NAA spectra were detected in female SMC (the strongest effect) and increased level of Glc spectra in female OFC. However, age-related differences were demonstrated due to increased levels of NAA, GABA, GABA+Glu, Glu, Gln, Ins, and Lac spectra in OFC (the strongest effect) and increased level of GABA+Glu spectra in SMC with advancing age. Although the specific physiologic roles of some neuronal and axonal chemical markers are currently unknown, glutamate and GABA are the major excitatory and inhibitory neurotransmitters of the brain and are released by approximately 90% of the cortical neurons and synapses (Shulman and Rothman, 1998; Magistretti *et al.*, 1999), and GABA and glutamate shifts compose the major contribution to the detected age and regional differences. Previous studies consistently showed an increase in relative concentration of NAA during the first 2 years of life up to 16 years of age (Peden *et al.*, 1990; van der Knaap *et al.*, 1990; Bruhn *et al.*, 1992; Toft *et al.*, 1994a, 1994b). This must be related to neuronal maturation and seems to be completed many years after birth. Our data suggest that NAA is age-dependent within the studied age groups, and increased NAA concentration is observed in OFC with advancing age. NAA is also sex-dependent, and increased level of NAA is found in female SMC. It is known that NAA is localized within neurons and involved in synaptic processes and can be considered a neuronal and axonal marker (Miller, 1991; Castillo *et al.*, 1998). Subsequent breakdown of NAA leads to aspartate, which is an excitatory amino acid neurotransmitter. Although understanding of the specific role and effects of each neurotransmitter on sex and age differ-



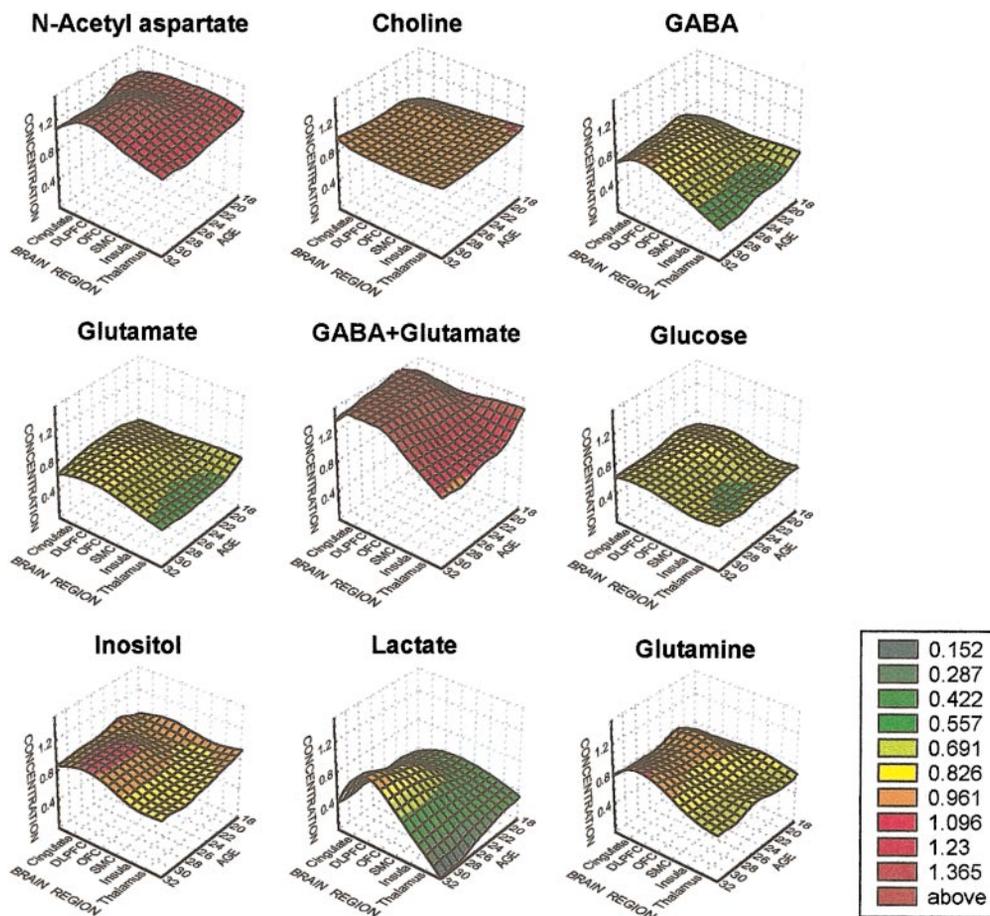
**FIG. 4.** 3D surface plot showing age-dependent differences in metabolite concentration for six regions of the human brain. The strongest interaction effect of Age on regional brain metabolism is in the OFC and SMC. Increases of metabolite concentration in OFC and SMC are demonstrated with advancing age from 19–20 to 25–31 years.

ences in human brain is still limited, numerous data demonstrate that sex hormones can regulate and interact with growth factors, neurotransmitters, neuropeptides, neuroactive steroids, and neuronal second messengers to influence neuronal differentiation, growth, and synapse formation (reviewed in Rubinow and Schmidt, 1996). Recent findings also suggest that several endocrine factors, including growth hormone, testosterone, and dehydroepiandrosterone, decline gradually with age (Rudman *et al.*, 1990). Overall, the different levels in regional metabolite concentration between the sexes and between age groups can be related to neuronal maturation such as increases in the number of axons, dendrites, and synaptic connections proceeding at different directions across brain regions in men and women and from 19–20 to 25–31 years of age. The most recent morphometric MRI study revealed the structural maturation of neural corticospinal and frontotemporal pathways in children and adolescents ages 4 to 17 years (Paus *et al.*, 1999). Our findings show functional neurochemical aspects of the neuronal maturation, suggesting that these processes may not be completely finished during adolescence and continue toward adulthood.

The observed regional and age- and sex-related effects on brain biochemistry overlapped mostly in the prefrontal regions and SMC. Given that the prefrontal cortex is implicated in working memory, planning and sequencing of behavior, language and attention (dorso-lateral part), and decision-making processes (ventromedial or orbital part) (Bechara *et al.*, 1994, 1997; Fuster, 1997; Damasio, 1998; Pandya and Yeterian, 1998; Smith and Jonides, 1999), our data provide further evidence that cognitive development of prefrontal cortex has chemical underpinnings. Since SMC is in-

involved in sensory and motor processing, perception, and motor execution, the exact mechanisms of sexual dimorphism and development in this area need to be further clarified.

The first evidence for sex and age differences in the learning performance of rhesus monkey whose orbital frontal cortex had been removed in infancy was described by Goldman-Rakic *et al.* (1974). It was found that male rhesus monkeys with orbital frontal lesions were impaired on behavioral tests at 2.5 months of age, whereas similar deficits were not detected in females with comparable lesions until 15 to 18 months of age. These results suggested that (1) the maturation of the orbital frontal cortex in the primate brain proceeds at different rates in males and females, (2) developmental disparities between the sexes might be found in other brain regions as well, and (3) similar sex differences might occur in the brains of humans. These ideas were explored in several postmortem and structural neuroimaging studies in humans which indicated that sex, age, and sex in aging differences exist in brain morphology (Allen *et al.*, 1989; Clark *et al.*, 1989; Witelson, 1989, 1991; Witelson and Kigar, 1992; Witelson *et al.*, 1995; Murphy *et al.*, 1996; Giedd *et al.*, 1997; Passe *et al.*, 1997; Kidron *et al.*, 1997; Coffey *et al.*, 1998; Gur *et al.*, 1999). The most recent structural MRI study by Gur *et al.* (1999) found that women have a higher percentage of gray matter, whereas men have a higher percentage of white matter and of CSF, and both gray and white matter volumes correlated with cognitive performance across sex groups. Earlier studies also show sex effects on human brain anatomy (Pakkenberg and Voigt, 1964; Geschwind *et al.*, 1968). Functional brain imaging studies (single-photon emission tomography and positron emission tomography (PET)) indi-



**FIG. 5.** Metabolite-specific age differences in concentration for six regions of the human brain. Age differences in the relative concentrations of *N*-acetyl aspartate, choline, GABA, glutamate, GABA plus glutamate, glucose, myo- and scyllo-inositol complex (inositol), lactate, and glutamine are presented in 3D surface plot. Significant increases in *N*-acetyl aspartate, GABA, glutamate, GABA plus glutamate, glutamine, lactate, and inositol concentrations in OFC, and GABA plus glutamate in SMC, are demonstrated with advancing age.

cate sex differences in brain metabolism, particularly in global and regional cerebral blood flow and glucose consumption (Gur *et al.*, 1982, 1995; Devous *et al.*, 1986; Baxter *et al.*, 1987; Rodrigues *et al.*, 1988; Yoshii *et al.*, 1988; Miura *et al.*, 1990; Murphy *et al.*, 1996). PET receptor characterization and pharmacological studies showed the ability to detect sex differences for some neurotransmitters and receptors. The mean rate of serotonin synthesis in normal males is 52% higher than in normal females (Nishizawa *et al.*, 1997). Significantly higher type-2 serotonin receptor binding capacity is found in men than in women, especially in the frontal and cingulate cortices (Biver *et al.*, 1996). In this study the brain metabolic and plasma catecholamine responses, after infusion of  $\alpha_2$ -adrenoceptor antagonists, showed global increases in metabolism in female subjects, whereas males had no global changes and some regional decreases in metabolism following  $\alpha_2$ -adrenoceptor blockage. Gender differences have also been detected in analgesic responses to  $\mu$ - and  $\kappa$ -like opioids (Gear *et al.*, 1996; reviewed in Mias-

kowski and Levine, 1999). Postmortem studies revealed that the levels of dopamine, norepinephrine, acetylcholine, and GABA are reduced with aging (reviewed in Edvinsson *et al.*, 1993). Significant age-related decrements in large cortical neurons and a reduction of cortical thickness have also been reported (Terry *et al.*, 1987). The total number of neurons in human neocortex was reduced by 10% over the life span in both sexes, and sex and age were the main determinants of the total number of neurons in neocortex that was determined using a modern stereological method (Pakkenberg and Gundersen, 1997). A PET study by Murphy *et al.* (1996) shows age-related decrements in global and lobal glucose metabolism as well as decrements in regional volumes.

In conclusion, our results provide evidence that the living human brain is chemically heterogeneous. The regional concentration of all studied metabolites are heterogeneous for different brain areas, in prefrontal regions markedly. The chemical heterogeneity is also sex- and age-dependent. It was determined that the *in*

*vivo*  $^1\text{H}$  MRS is sensitive enough to detect regional and age- and sex-related biochemical differences in the human brain. This study also confirms the importance of controlling for age and sex in MRS studies, particularly in clinical brain research. Although the present data are limited for several reasons, including small sample size, relatively narrow age range (ages 19–31), and measuring only one hemisphere (left), we suggest that these preliminary findings might support several possible hypotheses to be tested in future studies of sex- and age-related brain chemical differences in larger cohorts of subjects, as well as in right hemispheric regions, across a wider range of age groups. The correlation between performance in a specific frontal lobe task and frontal metabolites measured via MRS was suggested (Volz *et al.*, 1998). Future studies will be important for understanding whether the observed differences in the brain neurochemistry and the effects of sex, age, and brain region are dependent upon heterogeneity of cognitive development in the living human brain.

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