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Multi-chemical networking profile of the living human brain: potential relevance to molecular studies of cognition and behavior in normal and diseased brain

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Summary. Anatomical, electrophysiological and functional neuroimaging studies show that the human brain is a complex network, where cortico-cortical and thalamo-cortical connections are organized in a specific pattern giving rise to brain function. In our recent studies we found that chemical connectivity between brain regions might be changed in different conditions (e.g. aging, chronic pain, cognitive interference). The elucidation of properties of the human brain multi-chemical networking profile is the subject of this study. In vivo proton magnetic resonance spectroscopy was used to determine relative concentrations of multiple chemicals (N-Acetyl aspartate, choline, glutamate, glutamine, GABA, inositol, glucose, and lactate in relation to creatine/phosphocreatine complex) in 6 brain regions: thalamus, and cingulate, insula, sensorimotor, orbital frontal, and dorsolateral prefrontal cortices. The properties of the brain multi-chemical networking profile within and across the studied regions were examined using correlation analysis. Strong positive correlations were seen between chemicals within brain regions. Negative correlations were primarily seen across brain regions. The cortical connectivity for both neurotransmitters (GABA and glutamate) was stronger than for the other chemicals, and was stronger than for the same neurotransmitters in the thalamus. Factor analysis indicated that the natural clustering of regional chemical concentrations is by brain region and not by chemicals. These findings support the idea for the existence of a specific pattern of multi-chemical networking profile in the brain where the major excitatory and inhibitory neurotransmitters in neocortex perform a regulatory function.

Keywords: Human brain, multi-chemical networking profile, neurotransmitters, prefrontal cortex, sensorimotor cortex, thalamus, proton magnetic resonance spectroscopy.

Abbreviations

ANOVA analysis of variance, *Cho* choline, *Cr* creatine and phosphocreatine complex, *DLPFC* dorsolateral prefrontal cortex, *GABA* γ-Aminobutyric acid, *Glc* glucose, *Glu* glutamate, *Gln* glutamine, *^{1}H-MRS* proton magnetic resonance spectroscopy, *Ins* myo-inositol, *Lac* lactate, *MCNP* multi-chemical networking profile, *NAA* N-Acetyl aspartate, *OFC* orbital frontal cortex, *SMC* sensorimotor cortex.

Introduction

The history of research on the connectivity of the brain is almost as long as that of neuroscience research. Many anatomical, electrophysiological and functional neuroimaging studies show that the human brain is a complex network, where numerous cortico-cortical and thalamo-cortical connections are organized in a specific way giving rise to brain function (reviewed in Fuster, 1997; Pandya and Yeterian, 1998). By the application of modern methods of anatomy, mostly studied in non-human primates, there is now a detailed, although not yet complete, picture of the overall brain connectivity and neural circuits that link cortex and thalamus (Goldman-Rakic and Schwartz, 1982; Rakic, 1983; Goldman-Rakic, 1984, 1987; Kostovic and Rakic, 1984; Alexander and Crutcher, 1990; Kritzer and Goldman-Rakic, 1995; Romanski et al., 1997). Pharmacological studies have demonstrated a wide distribution of neurotransmitter receptors and their pathways in the brain (Levitt et al., 1984; Rakic et al., 1986, 1988; Lidow et al., 1989a,b, 1990, 1991a,b; Bergson et al., 1995; Mesulam, 1995, 1996; Selden et al., 1998; Williams and Goldman-Rakic, 1998; Perry et al., 1999). Dynamics of neuronal interactions in monkey frontal cortex in relation to behavioral events have been demonstrated using simultaneous recording of neuronal activity (Vaadia et al., 1995). Long-distance synchronization of human brain activity has been described using electroencephalography and magnetoencephalography (Llinas and Ribary, 1993; Tiitinen et al., 1993; Desmedt and Tomberg, 1994; Joliot et al., 1994; Pantev, 1995; Tallon-Baudry et al., 1997; Rodriguez et al., 1999). Network analysis of positron emission tomography (PET) study of regional cerebral blood flow show an ensemble inhibition during episodic memory retrieval (Nyberg et al., 1996). The cortical network has also been shown, using functional magnetic resonance imaging (fMRI), to be a dynamical process, which undergoes task dependent re-organization (Gelnar et al., 1999; Apkarian et al., 1999). However, further understanding of the brain as a large-scale neuronal network, and identification and quantification of the specific neurotransmitter mechanisms of the neuronal activity/metabolism defining the network can be achieved by examining human brain multi-chemical profile in vivo, using the method of magnetic resonance spectroscopy (MRS).

Previous studies indicate that (i) the human brain is a complex network, where numerous cortico-cortical and thalamo-cortical connections are organized in a specific way, (ii) glutamate and GABA are the major excitatory and inhibitory neurotransmitters of the brain, which are released by approxi-

mately 90% of the cortical neurons and synapses (Shulman and Rothman, 1998; Magistretti et al., 1999), and (iii) most long-distance connectivity (asymmetrical synapses) within the cortex, and most thalamocortical afferents and efferents that linked to pyramidal neurons are mediated through glutamatergic synapses (White, 1989). In our recent studies we found that chemical connectivity between brain regions might be changed in different conditions (e.g. aging [Grachev et al., 2001a], chronic pain [Grachev et al., 2000], cognitive interference [Grachev et al., 2001b], depression [manuscript in preparation]). The elucidation of properties of the human brain multi-chemical networking profile is the subject of this study. We tested the following hypotheses: A) The human brain is a specific multi-chemical network where different chemicals "communicate" in a specific way across brain regions, B) In this multi-chemical network organization, the excitatory (glutamate) and inhibitory (GABA) neurotransmitters compose the major contribution to the network interactions. The current report is the first where relationships between multiple chemicals within and across brain regions, which we identify as 'multi-chemical networking profile' (MCNP), is studied. Specifically we studied interrelationships between 9 chemicals, in six brain regions, in 19 young normal adults, using in vivo proton (^{1}H) MRS method (Fig. 1).

Materials and methods

Subjects

19 normal right handed volunteers (11 men and 8 women, mean age = 24.9 ± 2.4, age range 19–31) participated in the ^{1}H 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 signed a consent form. The Institutional Review Board approved all procedures in this study.

Localized in vivo 1H 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.

Global shimming. Global shimming is a procedure for optimizing the magnetic field homogeneity over the entire brain volume, as well as for each specific regional volume. Automated shimming has been used as a part of 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 Tesla General Electric (Signa) clinical imaging instrument. 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 are obtained with 6.0 mm slice thickness and a 0.5 mm gap between slices, and images are performed in 20 slice locations.

Selection of volume of interest. Localized ^{1}H MRS is then performed in 4 axial (thalamus, insula, orbital frontal cortex [OFC] and sensorimotor cortex [SMC]) and in 2 sagittal (cingulate and dorsolateral prefrontal cortex [DLPFC]) locations in the left hemisphere (dominant) of right-handed normal volunteers as described earlier (Grachev and Apkarian, 2000; Grachev et al., 2001a). Briefly, we used 8 cm^3 voxels for each

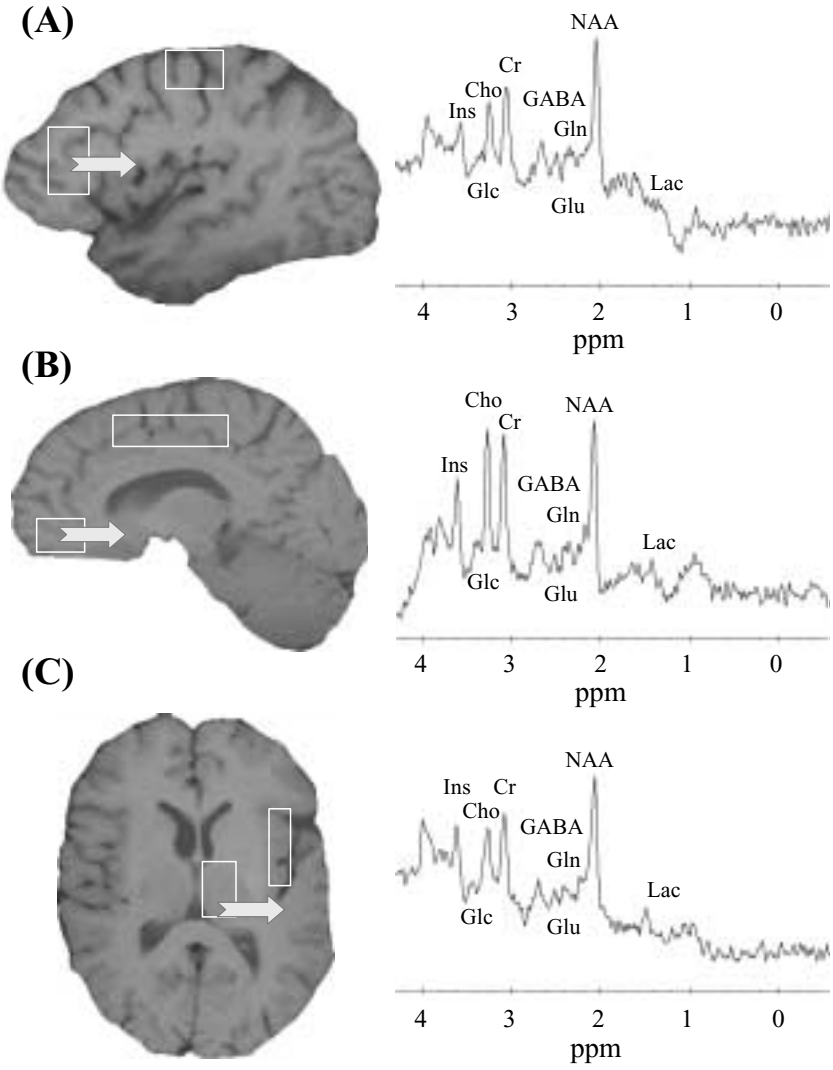

Fig. 1. An in vivo proton MRS brain methodology. T1-weighted spin echo high-resolution MR images showing the position of 6 brain voxels in the left hemisphere. The size and positioning of regional voxels for DLPFC (lower box) and SMC (**A**), OFC (lower box) and cingulate (**B**), and thalamus (lower box) and insula (**C**). Typical in vivo proton MR spectra (**A–C**) showing position of major peaks for NAA, Cr, Cho, and small peaks for Glu, Gln, GABA, Ins, Glc and Lac

analyzed volume: 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 × 1.6 × 1.0 cm for cingulate, and 2.0 × 2.0 × 2.0 cm for OFC (Fig. 1A–C). These boundaries were first identified on Talairach atlas (Talairach and Tournoux, 1988) using the following sections for regional voxel placement: horizontal +8 mm for the thalamus, horizontal +4 mm for the insula, horizontal +60 mm for SMC, horizontal −16 mm for OFC, sagittal 47 mm for DLPFC, and sagittal 5 mm for the cingulate; and then adjusted to the individual brain's sulcal topography (Grachev et al., 1999). These six regional volumes were selected arbitrarily. The right hemisphere was not studied because of time limitations. The scan time for each region was 6.5 min with a total scanning time for all regions of about 1 hour.

MRS data collection. Proton localized spectra were collected using a simulated-echo acquisition mode (STEAM) sequence (probe-s PSD, TR = 1,500 ms, TE = 30 ms, 256 averages). All spectra were collected from identical-sized voxels, which were specific for each regional volume.

MRS data processing. All spectra were transformed into a standardized scale using the Scion Image analysis package (Frederick, Maryland, 1998) and analyzed as described (Grachev and Apkarian, 2000; Grachev et al., 2001a). The relative concentrations of N-Acetyl aspartate (NAA), choline (Cho), glutamate (Glu), glutamine (Gln), γ-Aminobutyric acid (GABA), myo-inositol complex (Ins), glucose (Glc) and lactate (Lac) were measured relative to concentration for creatine/phosphocreatine complex (Cr), which is commonly used as an internal standard. Figure 1A–C shows typical proton MR spectra for three brain regions. Three major peaks usually characterize the ^{1}H MR spectra: 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. Cr spectrum is a combination of creatine and phosphocreatine (Michaelis et al., 1993). This peak was relatively stable across the studied subjects and used as an internal standard (see below). The proton Cho signal is a combination of Cho and Cho-containing compounds: Cho plasmogen, glycerophosphorylcholine, phosphorylcholine, cytedine-diphosphate-choline, acetylcholine, and phosphatidylcholine (Michaelis et al., 1993). The other observable chemicals that measured in our study were Glu, 2.35 ppm; Gln, 2.15 ppm; GABA, 2.25; Ins, 3.60 ppm; Glc, 3.43 ppm and Lac, 1.3 ppm (Fig. 1A–C). These smaller peaks are contaminated by signals from other chemicals and proteins, although the prominent signal is from the chemicals with which we identify these peaks (Salibi and Brown, 1998).

Limitations of peak ratio calculation. Several approaches have been used for computing peaks (see Salibi and Brown, 1998). The peak ratio method is currently used in clinical ^{1}H MRS mostly because it is simple and requires no technical expertise or software besides that supplied with the imager. The disadvantage of using ratios is that changes in either or both metabolite concentrations affects their ratio. In addition, both metabolites may vary in a way that leaves the ratio unchanged with respect to a "normal" value. Importantly, the use of a ratio where one chemical is measured simultaneously with all the others provides an internal normalization that would minimize variations due to technical confounders on the MRS measurements. We used the ratios method relative to Cr because the effect of subject on total Cr peak and on each regional Cr peak was non-significant ($F = 0.35$, $p = 0.99$). However, the coefficient of variation (CV) determined for each regional Cr across all studied subjects (CV = the standard deviation expressed as a percentage of the mean) showed regional variations: Cr in insula = 4.9%, Cr in thalamus = 6.9%, Cr in DLPFC = 7.8%, Cr in cingulate = 8.1%, Cr in SMC = 8.2%, Cr in OFC = 8.9%, and total Cr in all regions was 11%. Also, this approach is precise and accurate if the peak ratio is used for the assessment of normal subjects. Although, the peak/Cr relationships may be different for some brain disorders and in such cases it may be worthwhile to combine peak ratio measures with other approaches (e.g., absolute metabolite measurement relative to external or internal standards).

Reliability of the multi-chemical measurements. To compare the amount of variation in the measurement of multiple chemicals and their ratios, the CV was determined for each chemical. The CV determined by five repeat scans of one region of interest in a single scanning session on one normal subject showed excellent reliability. These measurements performed for the thalamus were as following: NAA/Cr = 2.5%, Cho/Cr = 4.0%, GABA/Cr = 6.6%, Glc/Cr = 3.4%, Ins/Cr = 2.0%, Lac/Cr = 8.5%, Glu/Cr = 6.5%, and Gln/Cr = 3.3%. A similar analysis for other brain regions also shows excellent reliability. The reliability study performed on the same subject during several weeks (five repeated sessions of six brain regions in an interval of one week) also shows excellent reliability (CV = 2.0–9.5%). These results were consistent with the data presented by Simmons et al. (Simmons et al., 1998), which measured chemical ratios from the same sized voxel prescribed in occipital lobe and repeated five times on eight normal subjects. The reliability study performed on the same subjects over 3 months only slightly worsened the results (Simmons et al., 1998).

Statistical analysis

Effect of brain region × chemical interaction was analyzed with analysis of variance (ANOVA), using the general linear model (STATISTICA, Tulsa, OK). The outcome variable was taken to be the chemical concentration. In this 2-way ANOVA, brain region, chemical, and subject were used as explanatory variables. Principal components factor analysis was performed on the data to determine the chemical variables that best cluster the 48 dimensional space (8 chemicals × 6 brain regions). Pairwise correlations (Pearson's Correlation Coefficient) were determined across the 48 dimensional data, to examine interrelationships between regional chemicals. A cutoff of correlation value of 0.5 was used since it results in a nominal threshold of $p < 0.05$. 2-way ANOVA was also used to distinguish between the correlation matrix properties. The latter analysis is used as a descriptive metric and not a random factor analysis.

Results

Analysis of variance

First, we performed a 2-way ANOVA to test whether ^{1}H MRS can detect brain region × chemical interaction across studied subjects. A 6 (brain region) × 9 (chemical) measures ANOVA, with concentration defined as the dependent variable, revealed a significant multivariate main effects for brain region [$F(5, 738) = 64.87$, $p < 10^{-7}$], chemical [$F(8, 738) = 411.03$, $p < 10^{-7}$], and for the brain region × chemical interaction [$F(40, 738) = 5.84$, $p < 10^{-7}$] (Fig. 2).

Factor analysis

Since the ANOVA found the brain region × chemical interaction, principal components factor analysis was used to detect a specific pattern of organiza-

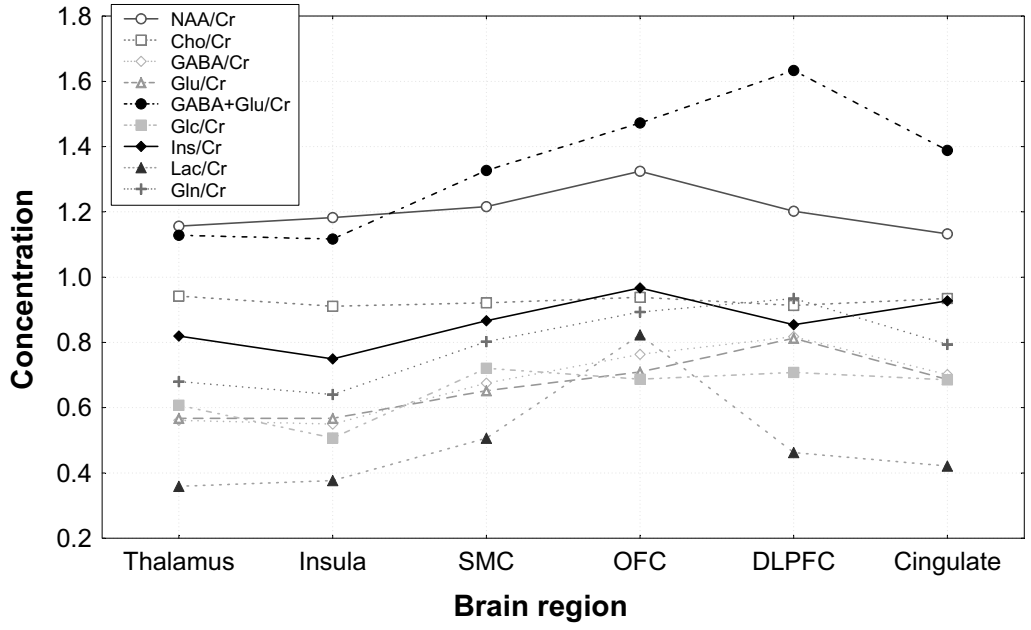

Fig. 2. Effects of the brain region × chemical interaction on the chemical concentration in the human brain

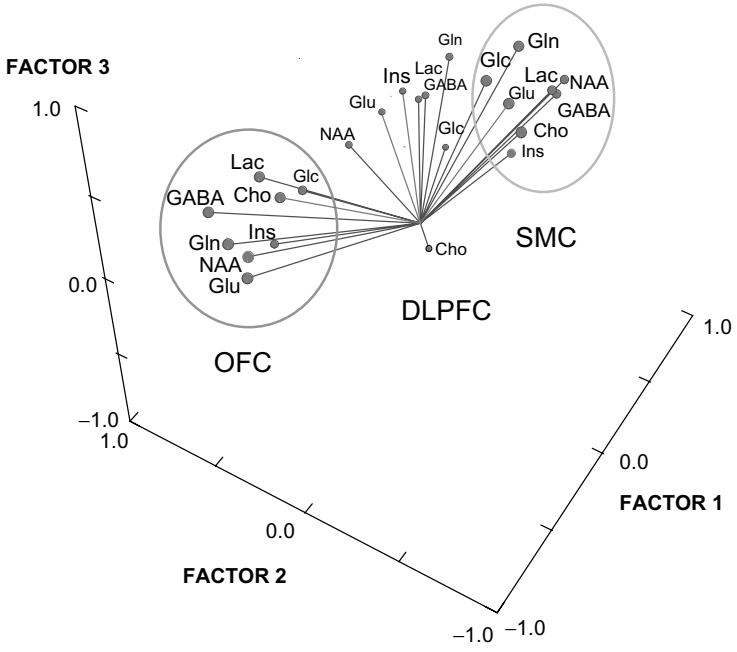

Fig. 3. Principal components factor analysis for the 8 metabolites across 3 brain regions. Clustering for metabolites in OFC (left circle), SMC (right circle) and DLPFC are shown

tion for concentrations of chemicals as a function of brain regions and chemical types. This analysis was performed for 8 chemicals (GABA + Glu was excluded since it is a linear combination of two chemicals) and 6 brain regions. Factor analysis indicated that the natural clustering across all 48 dimensions is by brain region and not chemicals. Figure 3 shows the results of factor analysis for 3 factors for all studied chemicals and for OFC, DLPFC, and SMC regions. The three factor loadings account for 74% of the variance. More importantly, each brain region occupies a specific quadrant within the 3-factor space. A similar analysis for the other three regions also clusters the data along brain regions.

Correlation analysis

Because the factor analysis indicates that chemicals are grouped by brain region, correlation analysis was used to determine the putative relationships between chemicals in and across brain regions (i.e., MCNP). Correlation analysis for the same 8 chemicals for 6 brain regions detected positive and negative correlations across 48 dimensions. The mean correlation strength for the two neurotransmitters (GABA + Glu) is shown across the 6 brain regions in Fig. 4. The strongest positive correlations were observed in DLPFC (mean $r = 0.85 \pm 0.09$), while the weakest positive correlations were found in insula (mean $r = 0.60 \pm 0.02$) and thalamus (mean $r = 0.59 \pm 0.02$). These correlation strengths for neurotransmitters parallel the concentration distribution across the brain regions studied (Fig. 2).

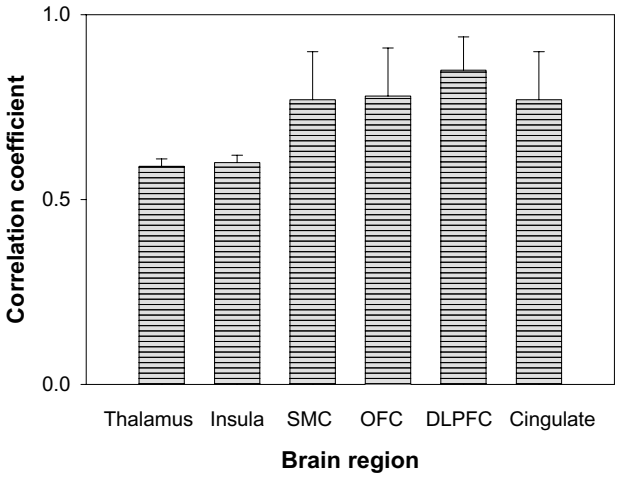

Fig. 4. The mean of correlation strength for GABA and glutamate (sum of two) within and across 6 brain regions

Intraregional correlations were primarily positive across all studied chemicals and brain regions (Table 1). Within a given region the number of positive correlations across all chemicals was highest in cingulate cortex and SMC. Only one negative correlation was seen in OFC. Interregional correlations were dominantly negative for cingulate, OFC and thalamus, and equally positive and negative for SMC, DLPFC and insula. A 2-way ANOVA of the incidence of significant correlations revealed a main effect for location (within a region vs. between regions) [$F(1,12) = 8.95$, $p < 0.01$], and the interaction between direction (positive vs. negative) and location [$F(1,12) = 22.43$, $p < 0.0005$]. The mean incidence of significant correlations when expressed relative to the total possible correlations were: for a given region 51% for positive correlations (mean = 14.25 of a total 28 possible), and 1% for negative correlations (mean = 0.25); and across regions 1% for positive correlations (mean = 9 of a total 820 possible), and 3% for negative correlations (mean = 23.5). Therefore, the dominant correlations occur within brain regions and are

Table 1. Number of positive ($r > 0.5$) and negative ($r < -0.5$) pairwise correlations across 8 chemicals and 6 brain regions

Brain Region	Intraregional		Interregional	
	Positive	Negative	Positive	Negative
Cingulate	28	0	18	38
SMC	25	0	11	17
OFC	11	1	4	32
DLPFC	15	0	17	21
Insula	16	0	24	20
Thalamus	6	0	4	24

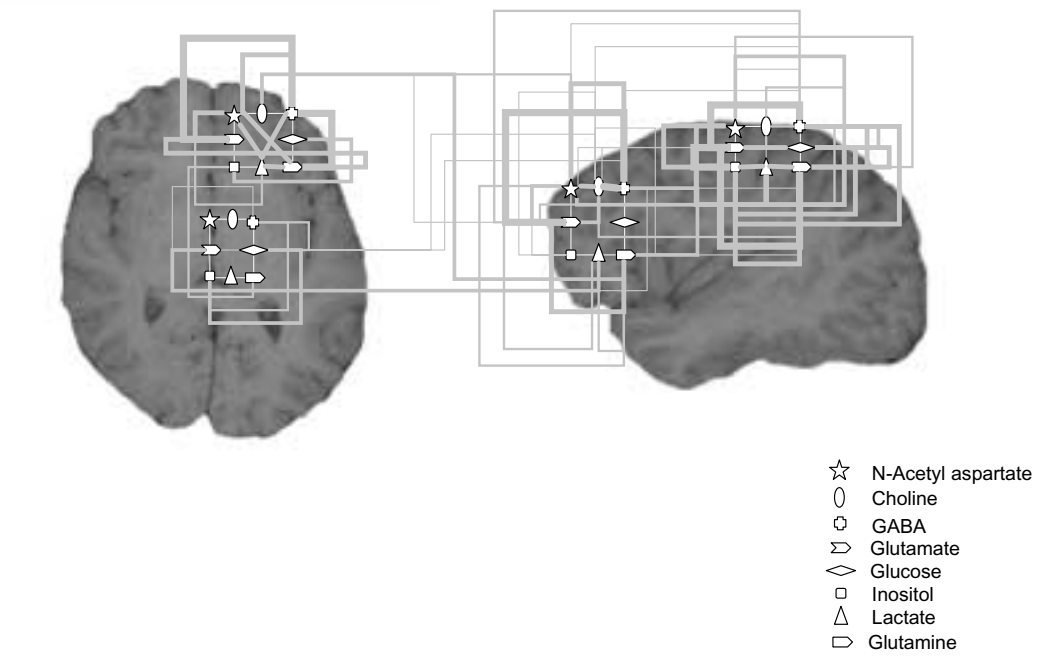

Fig. 5. Positive correlations for 8 metabolites across OFC, DLPFC, SMC and thalamus. The positive correlations are stronger between metabolites within each region, as compared to across regions. The number and strength of positive correlations are larger in cortical regions than in the thalamus. Lines indicate large positive correlations, and line thickness reflects strength of correlation between metabolites (0.75 pt correspond to $r = 0.50 - 0.59$, $p < 0.05$; 1.5 pt to $r = 0.60 - 0.69$, $p < 0.03$; 2.25 pt to $r = 0.70 - 0.79$, $p < 0.01$; 3 pt to $r = 0.80 - 0.89$, $p < 0.001$; and 4.5 pt to $r = 0.90 - 0.99$, $p < 0.00001$). Axial plane shows the location of ROIs for OFC and thalamus (lower box). Sagittal plane shows the location of ROIs for DLPFC and SMC (upper right box). Metabolites are presented as different geometric symbols, positioned on the ROIs

positive. To simplify the remaining analyses, only 4 brain regions are presented (OFC, DLPFC, SMC and thalamus), although the general organization was similar for the other two regions. Figure 5 shows the positive correlations for all 8 chemicals across OFC, DLPFC, SMC and thalamus. As seen on this schema, the intraregional correlations between chemicals are stronger than between chemicals across brain regions (mean $r = 0.76 \pm 0.13$ for intraregional correlations vs. $r = 0.61 \pm 0.07$ for interregional, $p < 10^{-7}$, t-test). Cortical correlations (within 3 regions) are stronger than thalamic correlations (mean $r = 0.77 \pm 0.13$ for cortex vs. $r = 0.66 \pm 0.10$ for thalamus, $p < 0.002$). The cortical connectivity for both neurotransmitters (GABA and Glu) was stronger than for the other chemicals. Across 5 cortical regions the mean correlation strength for GABA and for Glu with all other cortical chemicals was $r = 0.80 \pm 0.12$, as compared to the mean correlation for all other chemical interactions in the cortex ($r = 0.74 \pm 0.13$, $p < 0.05$). The cortical connectivity for both neurotransmitters was stronger than for the same neurotransmitters in the thalamus (mean $r = 0.66 \pm 0.10$ for thalamic GABA and Glu, $p < 0.0005$). The negative correlations detected a pattern

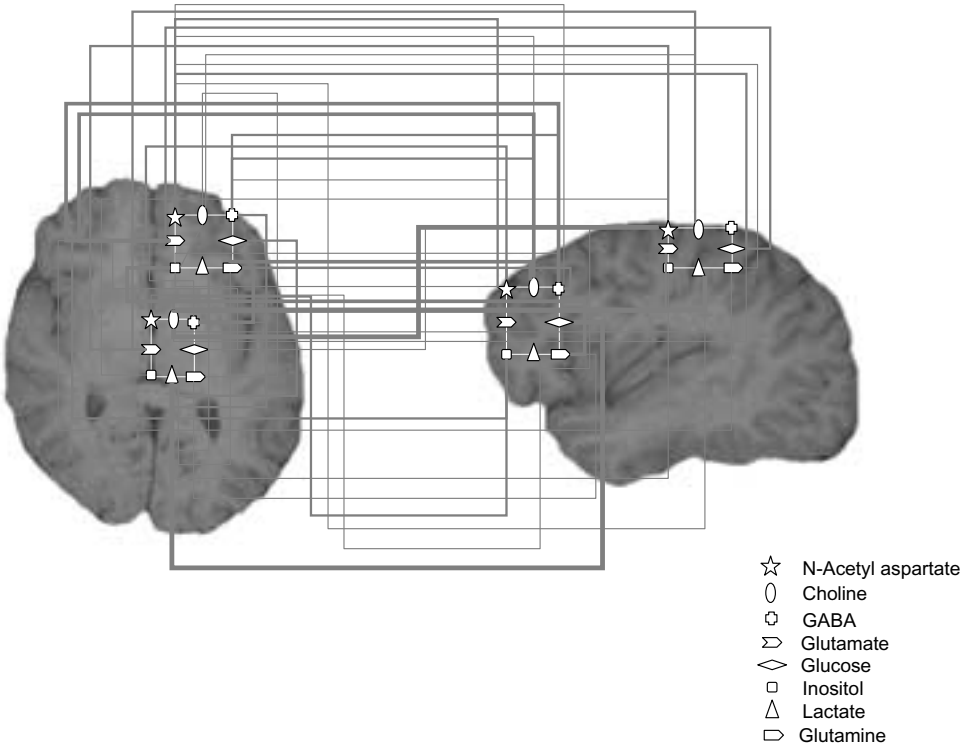

Fig. 6. Negative correlations for the 8 metabolites across OFC, DLPFC, SMC and thalamus. Negative correlations are primarily seen across brain regions. Lines indicate negative correlations and line thickness reflects strength of correlation between metabolites. For more details see legend of Fig. 5

different from the positive correlations (Fig. 6). Negative correlations are mostly seen across brain regions and are not seen between chemicals within each region. No negative correlations are observed between SMC and DLPFC chemicals, and the only intraregional negative correlation was in OFC between Ins and Cho. Although the total number of negative interregional chemical correlations was highest in cingulate cortex, OFC and thalamus, the strength of these correlations is not significantly different across all 4 regions, and the incidence of the total possible negative correlations was very low. There are no differences in the strength of negative correlations between cortical chemicals vs. thalamic, and between cortical neurotransmitters (GABA and Glu) vs. all other cortical chemicals, or thalamic neurotransmitters (all $p > 0.05$).

Discussion

Multi-chemical networking profile of the human brain

These results indicate that the living human brain is a chemically organized complex network, which can be characterized by MCNP. The patterns of MCNP are different for studied brain area (i.e., cortical vs. subcortical, and in

prefrontal regions) and brain region was found to be the main clustering parameter. We also detected a significant brain region – chemical interaction across studied subjects. Although the general hierarchy of cortico-cortical and thalamo-cortical connections have been demonstrated in numerous anatomical, electrophysiological and neuroimaging studies (Llinas and Ribary, 1993; Tiitinen et al., 1993; Desmedt and Tomberg, 1994; Joliot et al., 1994; Pantev, 1995; Nyberg et al., 1996; Fuster, 1997; Tallon-Baudry et al., 1997; Pandya and Yeterian, 1998; Gelnar et al. 1999; Rodriguez et al., 1999), the understanding of chemical interrelationships between the thalamus and the cortex, especially thalamo-prefrontal connections, and between different cortical regions have been unknown. In this study we present the MCNP between 6 brain regions, and between multiple chemicals within these regions. Strong positive correlations were seen between chemicals within brain regions. Negative correlations were primarily seen across brain regions. Different pattern of interchemical connectivity across brain regions, and predominance of positive intraregional correlations vs. positive interregional, and negative interregional correlations vs. negative intraregional, possibly reflects an integrative function of the human brain to preserve network specificity. It should be noted that this MCNP was determined for the left hemisphere of right-handed subjects. Given the functional differences between hemispheres, there may be important hemispheric differences in the multi-chemical network organization, which remains to be studied. Also, the MCNP is specified based on 8 cm^3 ^1H MRS voxels, which may cross Brodmann area boundaries. More detailed and specific information related to the MCNP properties can be obtained with smaller voxel sized ^1H MRS, which is just becoming available on newer MRI scanners.

The negative correlations were dominant for cingulate cortex, OFC and thalamus, and between them and other regions, which may reflect functional inhibitory reciprocal connections for these regions. This may underlie the theory of inhibitory control of interference (Fuster, 1997). There is substantial evidence that the control of interference is carried out by inhibitory counterinfluences originating primarily in OFC. The inhibitory OFC impulses can be processed through the thalamus to cingulate cortex, DLPFC and SMC. In this system cingulate cortex is important for controlling selective attention through suppressing the irrelevant and enhancing the relevant sensory information, which can explain the observed highest number of negative interregional correlations for this area. Reciprocal interference between OFC and DLPFC also can explain retention vs. suppressing of interfering memories (Fuster, 1997). However, the mechanisms of these chemical neuronal networks for processing cognitive and sensory information are still poorly understood. Certain metabolites and metabolite networks can reflect their involvement in specific higher-order brain functions. Nevertheless, to date we have little evidence for functional chemical specificity.

^1H MRS to study MCNP in normal and diseased brain

Recent advances in magnetic resonance imaging, and specifically in MRS, allow us to look inside of the energy metabolism of living brain cells and

perform measurements of regional brain chemicals. MRS is a powerful method to study living brain chemistry, which can be considered as a noninvasive biopsy of living tissue (Lock et al., 1990; Stanley et al., 1995). Modern MRS software imaging analysis programs measure regional concentrations of brain chemicals based on the differences in resonance frequency for different chemicals (Shulman and Rothman, 1998). In vivo ^{1}H MRS quantifies concentration levels of neurotransmitters such as glutamate and GABA, and the precursor for aspartate (NAA), 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). ^{1}H MRS also measures other chemical messengers and small molecules such as choline, glutamine, myo- and scyllo-inositol, glucose, lactate, creatine and phosphocreatine complex, which are involved in the metabolic pathway of tricarboxylic acid cycle, neuronal and astrocytic neurotransmitter cycling, and membrane turnover (Miller, 1991; Michaelis et al., 1993; Stanley et al., 1995; Gruetter et al., 1996; Salibi and Brown, 1998; Shulman and Rothman, 1998; Sibson et al., 1998; Magistretti et al., 1999).

Analysis of neurotransmitter systems in the brain and their network organization is important for understanding higher integrative functions and brain disorders. For example, degeneration of the dopaminergic system in the basal ganglia is linked with Parkinson's disease, while schizophrenia is associated with abnormalities of dopaminergic mechanisms in the basal ganglia and prefrontal cortex, and the cholinergic system is affected in Alzheimer's as well as in Parkinson's disease (Geula and Mesulam, 1996; Mesulam, 1996; Fuster, 1997). Using ^{1}H MRS, abnormal NAA concentrations were shown in epilepsy, schizophrenia, bipolar disorder, dementias, stroke, hypoxia, multiple sclerosis, leukoencephalopathies, and chronic back pain (Keshavan et al., 1995; Nasrallah et al., 1995; Stanley et al., 1995; Salibi and Brown, 1998; Grachev et al., 2000).

We found a significant increase of overall chemical correlation strength in MCNP within and across all studied brain regions with increased age (Grachev et al., 2001a). These changes were due to alterations in the pattern of negative chemical connectivity across brain regions, which become weaker (less negative) in middle-aged subjects as compared to young-aged. The interregional chemical connectivity for the cingulate cortex, SMC and the thalamus was changed the most with increased age (Grachev et al., 2001a). Increased levels of chemical correlation strength across brain regions in aging were found for most studied chemicals (including neurotransmitters GABA and glutamate), and not for NAA. Our most recent study of MCNP in chronic pain patients (as compared to age-matched normal subjects), using the same ^{1}H MRS approach, detected pain-related biochemical changes in the brain and abnormal MCNP across brain regions (DLPFC, cingulate and thalamus) (Grachev et al., 2000). Within brain regions multi-chemical connectivity decreased for most chemicals (mainly in DLPFC), and across brain regions connectivity increased (negative correlations became more negative), most likely as a result of long-term cortical reorganization. These findings already

demonstrate that ^{1}H MRS can be used for documentation not only of chemical concentration changes in the human brain, but also for assessment of specific patterns in MCNP in normal and diseased brains. Also, our findings should be interpreted cautiously due to less stable Cr levels in certain pathological conditions (see limitations of peak ratio calculation above). The approach opens a new avenue to study pathophysiological mechanisms of different neurological and psychiatric disorders.

Evidence for functional MCNP in the brain in relation to cognition

As was shown by Vaadia et al. (1995) correlated firing between single neurons, recorded simultaneously in the frontal cortex of monkeys performing a behavioral task, evolves within a fraction of a second, and in systematic relation to behavior. Their findings demonstrate that neurons can associate rapidly into a functional group in order to perform a computational task (positive correlations), at the same time becoming dissociated from concurrently activated competing more distant groups of neurons (negative correlations) to preserve the demarcation mechanisms. Long-distance synchronization of human brain activity in the frequency range 30–80 Hz (gamma oscillations) have been proposed as an integrative mechanism that may bring a widely distributed set of neurons together into a coherent cognitive act (Freeman, 1975; Damasio, 1990; Varela, 1995; Singer and Gray, 1995). Using recording of electrical brain activity from subjects who are viewing ambiguous visual stimuli (faces or meaningless shapes), Rodriguez et al. (1999) showed that local oscillatory responses can synchronize across multiple cortical regions, and the time course and topographic distribution of synchronization demonstrated a task-related specificity. These long-distance patterns of synchronization are involved in the processes of sensory perception and motor response, and the opposite process of desynchronization reflects an active uncoupling of the neural network that is necessary to proceed from one cognitive state to another (Varela, 1995; Rodriguez et al., 1999; Singer, 1999). As was proposed by Singer (1999) if the brain interprets responses as related when they are made synchronous by internal mechanisms, gamma oscillations could be the mechanism that binds neurons into functionally coherent assemblies. Our findings of widely distributed patterns of chemical connectivity (i.e., MCNP) in the human brain may reflect the chemical network organization of these binding mechanisms.

Neuroimaging studies also identified functional connectivity patterns in the brain involved in cognitive processes, mostly studied for episodic memory. Decreased activity patterns during episodic memory retrieval have been found, which was explained through inhibitory influences from other brain regions (Grasby et al., 1993; Andreasen et al., 1995; Nyberg et al., 1996). The network analysis of the pattern of interactions between brain regions demonstrated that influences from activated regions were stronger and more negative on regions showing relative deactivations in the retrieval condition, and the observed decreases in activity during episodic retrieval resulted from inhibition by brain regions showing increased activity (Nyberg et al., 1996).

Based on this network analysis, the concept of ensemble excitation and inhibition as a neural basis of episodic memory was introduced.

We did not study cognitive function in this study, which will be the next step in understanding of a larger scale of cognitive-MCNP patterns. However, recent ^{31}phosphorous-MRS study demonstrated correlation between performance in a specific frontal lobe task (Wisconsin Card Sort Test) and frontal high-energy phosphates (Volz et al., 1998). Biochemical markers of cognition (NAA) and intelligence (NAA and Cho) have been suggested using ^{1}H MRS (Jung et al., 1999a,b). Our most recent ^{1}H MRS study of cognitive interference in healthy subjects detected highly significant correlations between performance in the Stroop color-word task and the anterior cingulate NAA (Grachev et al., 2001b). Given that the prefrontal cortex is implicated in working memory, planning and sequencing of behavior, language and attention, and decision-making processes (Bechara et al., 1994, 1997; Goldman-Rakic, 1995; Damasio, 1998; Pandya and Yeterian, 1998; Smith and Jonides, 1999), our data provide further evidence that cognitive functioning of prefrontal cortex has chemical underpinnings that should be related to highest level of neuronal and synaptic connections, and functionally coherent long-distance patterns of synchronization in this brain region. Cingulate cortex is involved in selective attention, and SMC is involved in sensory and motor processing, perception and motor execution. The exact functional chemical mechanisms in which cingulate cortex and SMC are involved in a larger-scale of cognitive-chemical network needs to be studied. The precise nature of these chemical connections across the studied brain regions, organized in a more complex cognitive-chemical network, is unknown, and may be interfered with functional specificity of these brain regions. The specific pattern of cross- and intra-regional chemical bridging is needed to carry out multisensory and motor integration, and they most likely play an important role in the organization of complex human behavior and cognitive function. Our findings also can be considered as evidence of chemical organization for parallel neural networks (Hikosaka et al., 1999). According to this scheme, the connections between the cortical and subcortical regions are bi-directional, forming multiple loop circuits.

Neurotransmitters and MCNP properties

The specific pattern of MCNP in and across brain regions, described in our current study, is the indication, at the chemical level, of the widely associational nature of the human brain, which might reflect the distribution of different afferents and efferents between brain regions with which they are connected. In this scheme of neuronal pathway organization local multichemical changes may induce a distant effect. Our data further support previous reports that glutamate can be involved in excitatory effects, and GABA can be involved in inhibitory effects through the local regulation of glutamatergic receptors, possibly through disinhibition of excitatory neurotransmission in cortico-cortical and thalamo-cortical pathways.

The strongest positive relationships observed here were between cortical neurotransmitters studied (GABA and glutamate) vs. all other cortical metabolites, and between cortical vs. thalamic neurotransmitters. Moreover, the regional variation in mean concentrations of the neurotransmitters (GABA and glutamate) seems closely related to the regional variations in positive correlation strengths for these neurotransmitters. Glutamate and GABA are the major excitatory and inhibitory neurotransmitters of the brain, which are released by approximately 90% of the cortical neurons and synapses (Shulman and Rothman, 1998; Magistretti et al., 1999). Previous studies indicate that most long-distance connectivity (asymmetrical synapses) within the cortex, and most thalamocortical afferents and efferents that linked to pyramidal neurons are mediated through glutamatergic synapses (White, 1989). Our results show that GABA and glutamate connections compose the major contribution in the detected network interactions. Given the role of these neurotransmitters in millisecond changes in brain functional states, we propose that the regional neurotransmitter concentrations dictate the local metabolic demands. This local demand gives rise to the regional MCNP properties (positive correlations) that we observe between the neurotransmitters and the other local chemicals. Superimposed on this local metabolic control is a secondary interregional control mechanism for regulating metabolic demands, which is a competitive mechanism for distributing a limited source of energy. The latter mechanism would give rise to the negative correlations that we observe across brain regions. Moreover, the metabolic responses to these energy demands may be accomplished through metabolic pathways at multiple time scales, the properties of which remain to be studied.

In conclusion, in this report only six arbitrarily chosen left-brain regional volumes were presented. Thus, we need to know the overall picture of the whole brain MCNP patterns, as well as contribution of GABA and glutamate in the complex chemical orchestra that is engaged in the specific cognitive and behavioral performance. It is well established that GABA and glutamate are two major inhibitory and excitatory neurotransmitters in the human brain. A recent study by Sutoo et al. (2000) presented widely distributed systems of GABAergic and glutamatergic neurons in normal human brain using immunohistochemical fluorescence method. The real chemical networks (MCNP) in the brain that are involved in complex cognitive and behavioral functions are much more complex than described here. These studies are extremely intriguing and may have potential relevance to molecular studies of cognition and behavior in the living human brain. Regardless the interpretation of our findings, we have evidence from our ^{1}H MRS study to support earlier reports that all information concerning the environment, cognitive functioning and complex behavioral acts is transmitted within the brain by chemical transactions at synaptic junctions (e.g., axoaxonic or axodendritic). This is the way and place where the net of neurons is communicated. Future hypotheses also should be focused on the MCNP patterns in relation to behavior and cognition, for normal and diseased brain.

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