

## Appendix A14.24

### Non-Invasive Brain Neurochemistry

Over the past decade there have been rapid advances in the development and application of magnetic resonance spectroscopy (MRS) to study complex neuropsychiatric disorders (reviewed in Glitz et al., 2002; Moore and Galloway, 2002). MRS is a tool which provides a non-invasive window to brain neurochemistry; here, we briefly outline the essential principles of MRS, to provide an overview of some of the major neurochemical compounds observed in the brain via MRS and their potential significance in the study of recurrent mood disorders. Since the majority of technical development and clinical research findings in MRS in the last 5 years has been in the area of proton ( $^1\text{H}$ ) MRS, we will primarily focus our discussion around techniques, compounds and findings related to this nuclei.

Unlike MRI which provides high resolution images of brain anatomy primarily using signals from brain water and lipids, proton MRS actually suppresses these high background signals in order to measure the concentration of the major brain neurotransmitters and metabolites. Compounds which can be measured include N-Acetyl-Aspartate (NAA), Creatine/Phosphocreatine (Cr), Choline compounds (Cho), Myo-inositol (mI), Glutamate/Glutamine/GABA (Glx), and Lactate (Lac). Each of the individual compounds are identified by their peak position (or chemical shift) on the x-axis which is a frequency scale measured in parts-per-million (ppm). The area under each of the peaks (sometimes referred to as resonances) is proportional to the concentration of the neurochemical compound within the particular volume of the brain being investigated. The major MRS visible neurochemical compounds in the human brain and their potential significance briefly are briefly reviewed below.

#### **N-Acetyl-Aspartate (NAA) 2.02 Parts Per Million (ppm)**

This resonance is usually the most predominant peak in the normal brain and consists predominantly of NAA but also contains smaller contributions from other N-acetyl compounds including N-acetyl-aspartyl-glutamate (reviewed in Glitz et al., 2002; Moore and Galloway, 2002). While the functional role of this amino acid has not been determined, NAA is a putative neuronal marker (Tsai and Coyle, 1995), localized to neurons and not found in mature glial cells, CSF, or blood. A relative decrease in this compound may reflect decreased neuronal viability, neuronal function, or neuronal loss.

#### **Glutamate/Glutamine/ $\gamma$ -aminobutyric Acid (Glx) 2.3ppm**

The broad resonance centered at approximately 2.3ppm contains several overlapping

resonances predominantly glutamate and glutamine which are both involved in a glutamatergic pathway (reviewed in Glitz et al., 2002; Moore and Galloway, 2002).  $\gamma$ -aminobutyric acid (GABA) is a major neurotransmitter and is MR visible to a lesser extent due using conventional techniques due to its strong coupling properties, however spectral editing methods can be utilized to measure this compound specifically. Glutamate is a neurotransmitter and is the most abundant amino acid in the human brain and glutamine is believed to be localized primarily to cerebral astrocytes and is the primary derivative for glutamate. Elevated levels of the Glx region of the spectrum (particularly glutamate) are thought to be deleterious to neuronal tissue.

### **Creatine/Phosphocreatine (Cr) 3.02ppm**

This single resonance contains both creatine and phosphocreatine. Creatine is converted to phosphocreatine through the enzyme creatine kinase. Phosphocreatine is a high-energy phosphate which is critical for maintaining cellular energy dependent systems. The Cr peak has been used by a number of investigators as an internal standard for interpretation of qualitative changes in the concentration of the other MR visible neurochemical compounds and as such one often will see various references containing proton MRS data with Cr in the denominator (i.e.: NA/Cr or Cho/Cr). This reflects the assumption that the Cr resonance is relatively unaffected by various pathologies, this has yet to be definitively established. Potential pharmacological effects on Cr levels further confound the use of this resonance as a reference.

### **Choline Compounds (Cho) 3.23ppm**

The Cho resonance contains contributions from a number of mobile choline compounds, but predominantly from phosphorylcholine (PC) and glycerophosphorylcholine (GPC) and it therefore thought to be an important marker of membrane phospholipid metabolism. Other choline compounds such as free choline and acetylcholine contribute substantially less to this resonance (less than 5%). Tightly bound membrane choline compounds are generally thought to be MR invisible, however phosphatidylcholine (PTC) molecule does have a mobile head group with may render it partially MR visible. In disease processes which result in membrane breakdown, formerly bound choline is released into the free choline pool and becomes MR visible, which is thought to contribute to an increase of this resonance in neurodegenerative states. Shifts from intracellular to extracellular choline pools may also be a factor in observed choline changes due to differing MR visibility factors (reviewed in Glitz et al., 2002; Moore and Galloway, 2002).

### **Myo-inositol (mI) 3.56ppm**

The mI resonance contains predominantly myo-inositol with minor contributions (<5%) from glycine and inositol -1-phosphate. Myo-inositol is involved in osmoregulation of the brain

and is involved in the metabolism of membrane bound phospholipids. It is also directly involved in a number of important neuronal signaling systems including the phosphoinositide pathway. Perturbations in brain mI concentrations can have significant clinical effects on each of these systems. While there are reports of mI being a glial cell marker, high levels of mI are also present in some types of neurons (reviewed in Glitz et al., 2002; Moore and Galloway, 2002).

### **Lactate (Lac) 1.33ppm**

The lactate resonance appears in a region of the spectrum which often contains overlapping signals from lipids and macromolecules. Lactate is a product of anaerobic glycolysis and has a doublet peak structure centered at 1.33ppm with a 7Hz peak splitting. This compound is usually not present at MRS detectable concentrations (<0.7mM) in the normal brain. The presence of lactate in a region of the brain is often associated with pathology, seizure activity, or intense cognitive activation tasks (reviewed in Glitz et al., 2002; Moore and Galloway, 2002).

## **Some Essentials of MRS**

### **Common MRS Pulse Sequences**

The two most common pulse sequences used for the acquisition of clinical proton MRS studies are Stimulated-Echo Acquisition Mode (STEAM) and Point Resolved Spectroscopy (PRESS) (reviewed in Glitz et al., 2002; Moore and Galloway, 2002; Didwadkar and Keshavan, 2002). The STEAM sequence is based on a stimulated-echo methodology which uses 3 consecutive 90 degree radiofrequency (rf) pulses. The PRESS sequence is based on a double spin-echo rf scheme ( $90^\circ$ - $180^\circ$ - $180^\circ$ ). Both of these methods are most typically applied in single voxel (volume element) mode, however spectroscopic imaging methods based on these and similar techniques are now gaining widespread application. Acquisition parameters commonly utilized for visualizing brain neurochemistry are an echo time (TE) of 30 msec and a repetition time (TR) of 2 sec in order to maximize signal to noise of the compounds and minimize relaxation artifacts.

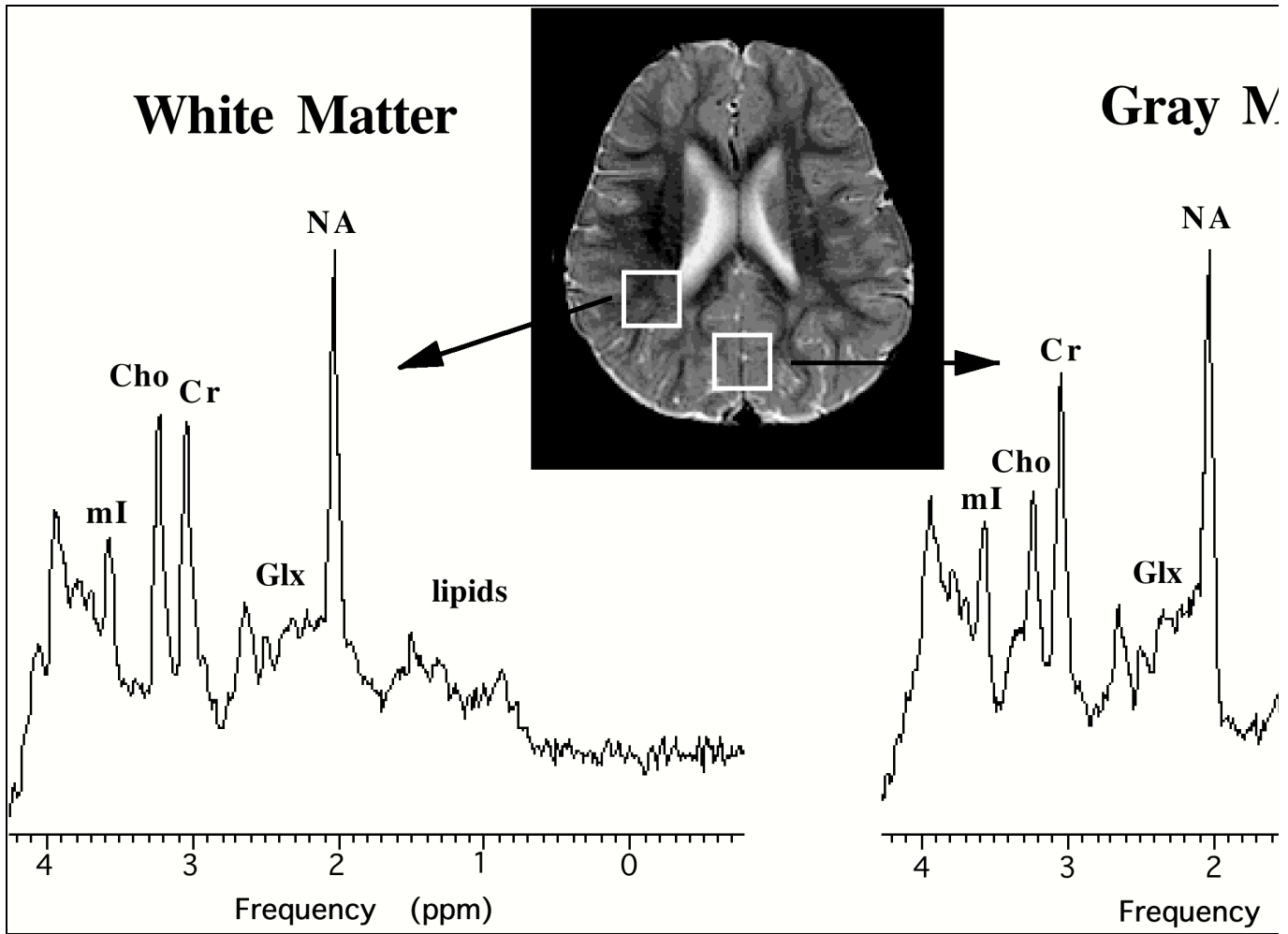


Figure A14.24a  
 Proton MR spectra from brain white and gray matter regions in a normal subject, TE=30/TR=2000. (NAA) N-acetyl-aspartate, (Cr) creatine/phosphocreatine, (Cho) choline compounds, (mI) myo-inositol, (Glx) glutamate/glutamine

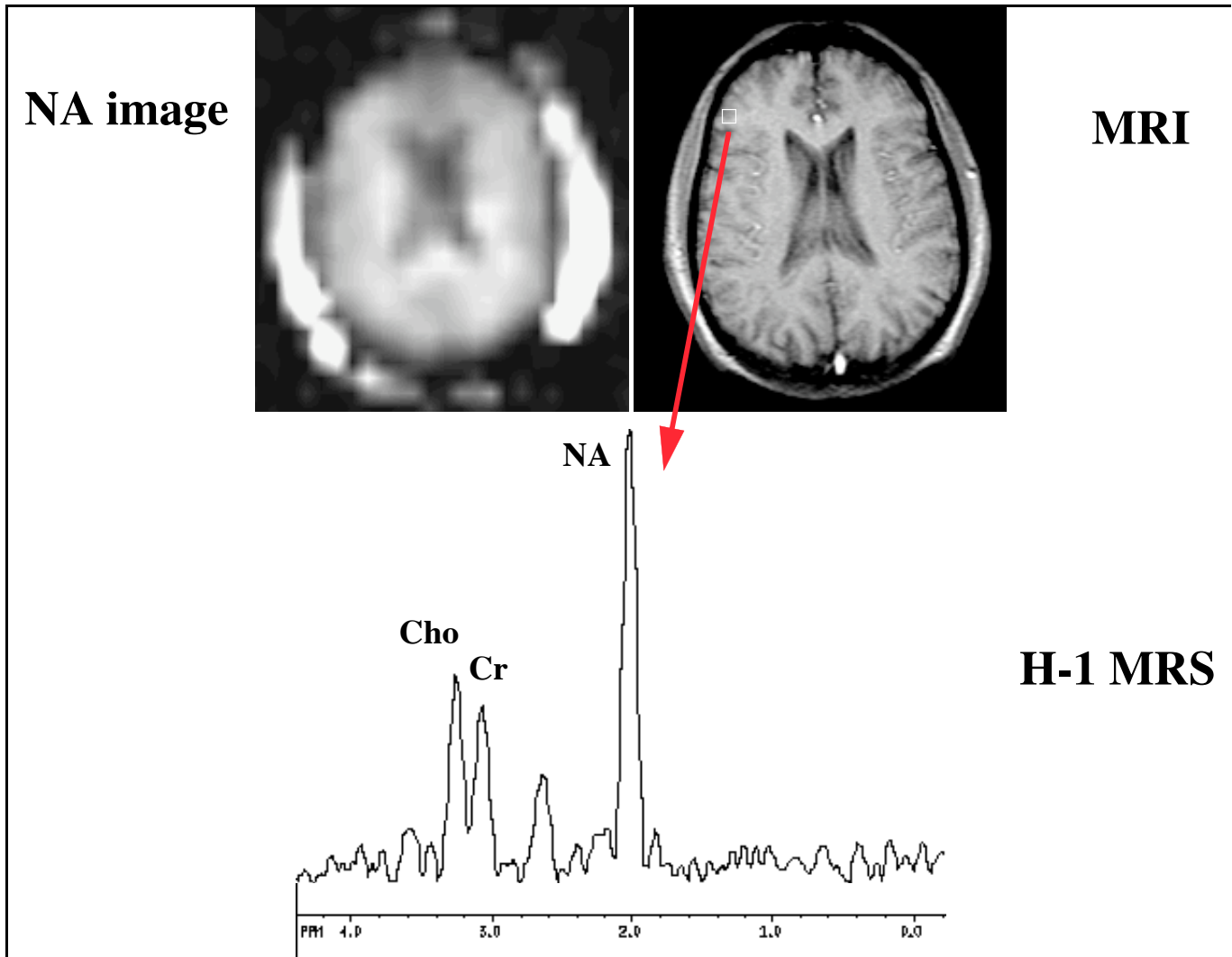


Figure A14.24b

Example of proton spectroscopic imaging study (TE=280/TR=2300). A map of the distribution of NAA throughout the brain in this normal subject is shown in this high resolution study. Individual spectra can also be extracted for quantitation from these data sets as illustrated.

### Neurochemical Quantitation

There are several approaches that may be utilized for quantitation of proton MRS spectral data however the two most commonly utilized are brain water referencing and internal brain neurochemical referencing. The water referencing approach uses brain water as an internal standard and assumes constant brain water content. This method has been utilized for over a decade for quantitation of in vivo MRS data. The internal brain neurochemical referencing approach is similar to the water referencing approach but instead assumes a constant

neurochemical compound concentration, most commonly Cr. The last approach is more qualitative in nature and makes use of ratios (usually with Cr in the denominator). The most common method utilized in the older literature is the later, however there is a consensus forming in the community that a more quantitative approach utilizing the brain water referencing method or related methods may ultimately prove to be more sensitive and specific for diagnosis than the more qualitative ratio methodology. This is especially the case when investigating pharmacological induced neurochemical changes as one cannot confidently assume that the neurochemical in the denominator (such as Cr which is involved in high energy phosphate metabolism) is not changing. There are several good software packages currently available for quantitative analysis of MRS data including MRUI and LCModel (reviewed in Glitz et al., 2002; Moore and Galloway, 2002). A final issue in quantitation of brain neurochemistry is the need to correct for partial voluming of different tissue types within the MRS volume of interest (gray matter, white matter, and CSF) as each component has a different baseline neurochemical composition. Again software packages such as MedX (Sensor Systems, Sterling, VA) are available for the purpose of MRI based tissue segmentation.

### **Magnetic Resonance Spectroscopic Approaches to Study Neuronal: Glial Interactions**

Previous studies indicate that glutamate released by the neuron is taken up by the surrounding glia and converted to glutamine by glia-specific enzyme glutamine synthetase. The inactive glutamine is then transported from the glia into the ECF where it is taken up again by neurons and converted back into glutamate via phosphate-activated glutaminase (PAG). This pathway is generally known as the glutamate-glutamine cycle between neurons and glia. *In vivo* magnetic resonance spectroscopy (MRS) is a noninvasive technique for the measurement of the concentration and synthesis of metabolites in the brain. Application of the state-of-the-art *in vivo*  $^{13}\text{C}$  and  $^{15}\text{N}$  MRS techniques to studying the synthesis of glutamate and glutamine in the human brain was reviewed in this paper with an emphasis on the validation of the measurement of the rate of the glutamate-glutamine cycle using different labeling strategies and using animal models.

Since the large glutamate pool, which is in fast exchange with  $\alpha$ -ketoglutarate, acts as a trap for  $^{13}\text{C}$  labels entering the TCA cycle from  $1\text{-}^{13}\text{C}$ -glucose, the time-course of  $4\text{-}^{13}\text{C}$  glutamate can be used to determine the rate of largely neuronal oxidative glucose metabolism. The subsequent label incorporation into  $4\text{-}^{13}\text{C}$  glutamine is closely related to the rate of glutamine synthesis and the rate of glutamate-glutamine cycle between neurons and glia. This allows extraction of the glutamate-glutamine cycle rate from the kinetics of  $^{13}\text{C}$  label incorporation from  $1\text{-}^{13}\text{C}$  glucose into the C-4 position of glutamate and glutamine. This measurement can be validated independently using other labeling schemes. For example, in the case of  $2\text{-}^{13}\text{C}$ -glucose,

the <sup>13</sup>C labels incorporate into the C-3 position in neuronal glutamate via the glutamate-glutamine cycle while the pathway used by 1-<sup>13</sup>C glucose can not bring <sup>13</sup>C label into non-carbonyl positions from 2-<sup>13</sup>C glucose. In the case of 2-<sup>13</sup>C acetate, which enters into the TCA cycle exclusively through glia, the labeling of C-4 position of glutamate is also via the glutamate-glutamine cycle. The glutamate-glutamine cycle can also be measured using <sup>15</sup>N MRS because <sup>15</sup>N can be incorporated into the amide position in glutamine and the amine position in both glutamate and glutamine. In all MRS studies the glutamate-glutamine cycle between neurons and glia was consistently found to be a major metabolic pathway with a flux rate in the range of 60-80% relative to neuronal oxidative glucose metabolism in the resting human cerebral cortex (adapted from Shen and Rothman, Magnetic resonance spectroscopic approaches to study neuronal:glial interactions, Biol. Psychiatry 2002, in press).

Table A14.24b

Summary of some recent key MRS findings in Mood Disorders

Neurochemical	Diagnosis	Finding
NAA	BP	decreased bilaterally in the hippocampus, and prefrontal cortex, chronic lithium Tx increases cortical levels
Choline Compounds	MD, BP	elevated in basal ganglia, negatively correlated with mood, decrease or increase with SSRIs, decrease with Li Tx
Myo-Inositol	BP	decreased in frontal lobe with Li Tx, negatively correlated with mood
Glutamate	MD	reduced in the anterior cingulate
GABA	MD	decreased cortical levels
Lithium	BP	brain Li levels may be a good predictor of Tx response
Fluorinated SSRIs	MD	fluoxetine accumulates in brain, brain levels are correlated with Tx response
Phosphocreatine/ATP	MD,BP	decreased brain levels
Phosphomonoesters,	MD,BP	abnormal levels, phospholipid metabolism compared

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Phosphodiesterases		to controls
pH	MD,BP	low brain pH compared to controls

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Abbreviations: MD, Major Depression; BP, Bipolar Disorder; TX, Treatment. Derived from Glitz et al., 2002; Moore and Galloway, 2002.

### References

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