CWave: Software for the Design and Analysis of ^{13}C Labeling Studies Performed *In Vivo*

Graeme F. Mason
Departments of Psychiatry and Biomedical Engineering, Yale University, School of Medicine, New Haven, CT, USA

Introduction ^{13}C MRS measurements yield metabolic rates and relative rates of substrate consumption based on the appearance of labeled substrates and products in the brain. From the time course of appearance of label, kinetic constants of transport and/or metabolism can be derived using the principles of mass and isotope balance. With costs of label and labor for cell cultures, animals, or human subjects, it is desirable when possible to obtain kinetic as well as steady-state data to obtain absolute metabolic rates and a larger number of rates than is obtained from only steady-state of the data. CWave was designed to simulate hypothesized metabolic flows to help users design studies and adjust parameters of metabolic schematics to match isotopic labeling data.

Software Design and Use CWave runs under Window 95, 98, and NT. From the main window of CWave the user defines and adjusts metabolite concentrations, rates of flow, kinetic constants, and time course data to define metabolic simulations. Once simulations are set up, they can be saved and later loaded from disk. From the Run menu, data can be fitted as fractional enrichment, ^{13}C concentration, and total (^{13}C + ^{12}C) concentrations.

Rates. Metabolic rates and kinetic constants can be entered numerically or as algebraic expressions that are evaluated when CWave runs the time course calculations.

Metabolites. Metabolites are defined either numerically or as algebraic expressions. Rates of output flows and input flows from user-designated substrate sources can be defined.

Driver Functions. Because for most studies *in vivo* the enrichments and concentrations of labeled substrates in the plasma vary among experimental preparations and from one subject to another, CWave accepts arbitrary input functions of labeled substrates.

Isotopomers and Resonance Overlap. Because the resonances of many metabolites *in vivo* are incompletely resolved, including some cases of isotopomer satellites with the singlet center peak, a utility is provided to include the effects of the overlap in the simulated time courses.

Fitting Procedures. Simplex fitting and simulated annealing are the choices for fitting time course data. Simulated annealing is generally slower but more effective when many parameters are being determined simultaneously.

Statistical Analysis. Time course studies allow evaluations of inter-subject variation through calculation of distributions of uncertainty for individual data sets. Non-negligible noise levels typical in MRS lead the individual error distributions to be non-normally distributed, frequently with tails toward high values. CWave uses a Monte-Carlo procedure (Mason et al., 1992, 1995) to evaluate uncertainty in individual time courses.

Application Data were analyzed for a study of hyperammonemia, pyruvate carboxylase, and glutamate-glutamine cycling (Sibson et al., 1999). Fig. 1 shows the fit of a model of glial and neuronal metabolism (Fig. 2) to glutamine C4 labeling data observed in a hyperammonemic rat in a study by Sibson et al. (1999) (glutamate C4 was also fitted but is not shown). The lower center of Fig. 1 also shows the distribution of uncertainty of the calculated diluting flux that flows into lactate (VdilLac).

References

1. Sibson et al. (1999) *Proc ISMRM*, p. 618
2. Mason et al., (1992) *J Cereb Blood Flow Metab* 12: 434
3. Mason et al. (1995) *J Cereb Blood Flow Metab* 15: 12

Acknowledgments

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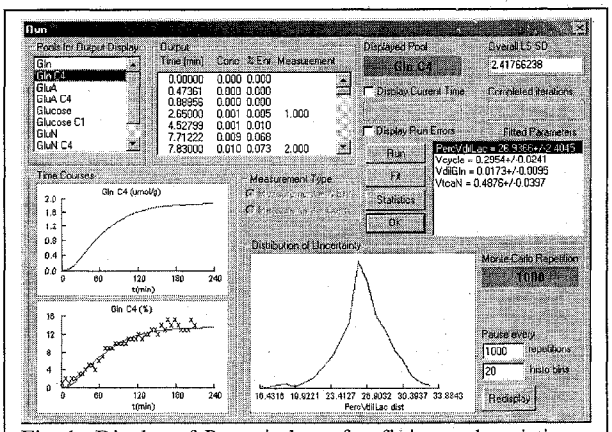

Fig. 1. Display of *Run* window after fitting and statistics.

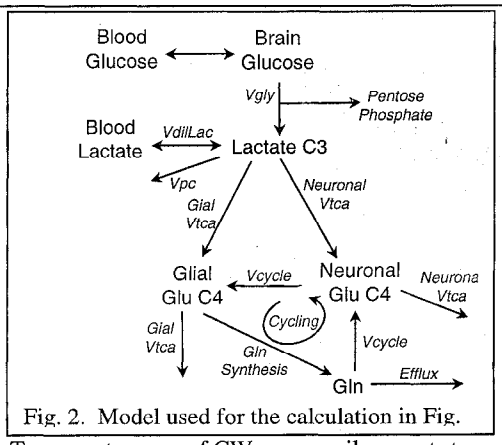

Fig. 2. Model used for the calculation in Fig.

To request a copy of CWave, e-mail requests to graeme.mason@yale.edu or mail to Graeme Mason, 138 Magnetic Resonance Center, 333 Cedar St., Yale School of Medicine, New Haven, CT 06520-8043