COMPARISON OF *IN VIVO* AND *IN VITRO* ^{1}H NMR SPECTROSCOPY OF RAT BRAIN: Technical Considerations, Effects of Brain Regions and Post-weaning Isolation.

Philippine C. Geiszler[1,2,*], A. Napolitano[1], M.I. Schubert[1], C.A. Jones[3], K.C.F. Fone[3], C.A. Daykin[2], D.P. Auer[1].

[1] Division of Academic Radiology, School of Clinical Sciences, University of Nottingham, Nottingham, NG7 2UH, UK; [2] Division of Molecular and Cellular Science, School of Pharmacy, University of Nottingham, Nottingham, NG7 2RD, UK; [3] School of Biomedical Sciences, University of Nottingham, Nottingham, NG7 2UH, UK. *msxpcg@nottingham.ac.uk.

INTRODUCTION.

Post-weaning isolation leads to some behavioural characteristics similar to those observed in psychiatric disorders, such as schizophrenia. Some neurochemical effects of isolation rearing are reported but need further investigation (Fone and Porkess, 2008).

In vivo and *in vitro* magnetic resonance (MR) spectroscopy are both used to obtain valuable, complementary information about the metabolic state of living tissue and tissue extracts, respectively. Previously, *in vivo* and *in vitro* concentrations of choline- and creatine-containing compounds showed good agreement, but *in vitro* N-Acetylaspartate levels seemed to be lower (Barker et al., 1994, Petroff et al., 1995, Tracey et al., 1996). However, comparisons between *in vivo* and *in vitro* measurements are rare.

Aim. The aim of this study was to compare results obtained from *in vivo* and *in vitro* MR spectroscopy to study inter-regional variations and the effects of social isolation on metabolite levels in rat brain.

METHODS.

***In Vitro* MR Spectroscopy.** Spectra were recorded from methanol extracts (Wu et al., 2008) of the frontal cortex and hippocampus from 19 isolation-reared and 27 group housed Lister-Hooded rats using a NOESY pulse sequence on a 400 MHz spectrometer (Bruker).

***In Vivo* MR Spectroscopy.** Spectra were acquired from a 3x3x3 mm^3 voxel in the frontal cortex and hippocampus of 8 isolation- and 8 group-reared Lister-Hooded rats using a ^{1}H Point-Resolved Spin echo sequence with a 7-T scanner (Biospec, Bruker) and analysed (jMRUI, LCModel).

Data Processing. Resonances corresponding to myo-inositol (mIns), taurine (Tau), choline-containing compounds ((G)PChol), aspartate (Asp)*, glutamine (Gln), glutamate (Glu), NAA, γ-aminobutyric acid (GABA), acetate (Acet)*, glycine (Gly)*, alanine (Ala)* and lactate (Lact)* were integrated (AMIX, LCModel) and related to the signal of creatine-containing compounds (Cr). The up-field area (0.3–4.3 p.p.m.) of *in vitro* spectra were also binned into 0.04 p.p.m. wide buckets, integrated (AMIX) and normalised to the sum of this area. * resonances were only quantified from *in vitro* spectra.

Statistical Analysis. Multivariate analysis, *i.e.* PCA, PLS, PLS-DA and PC-DA, was performed on all mean-centred data sets (PLS toolbox). Univariate analysis, *i.e.* Mann-Whitney-U tests and Spearman correlation, was used for group comparisons and correlation analyses of single metabolites (MATLAB).

RESULTS.

^{1}H NMR Spectra.

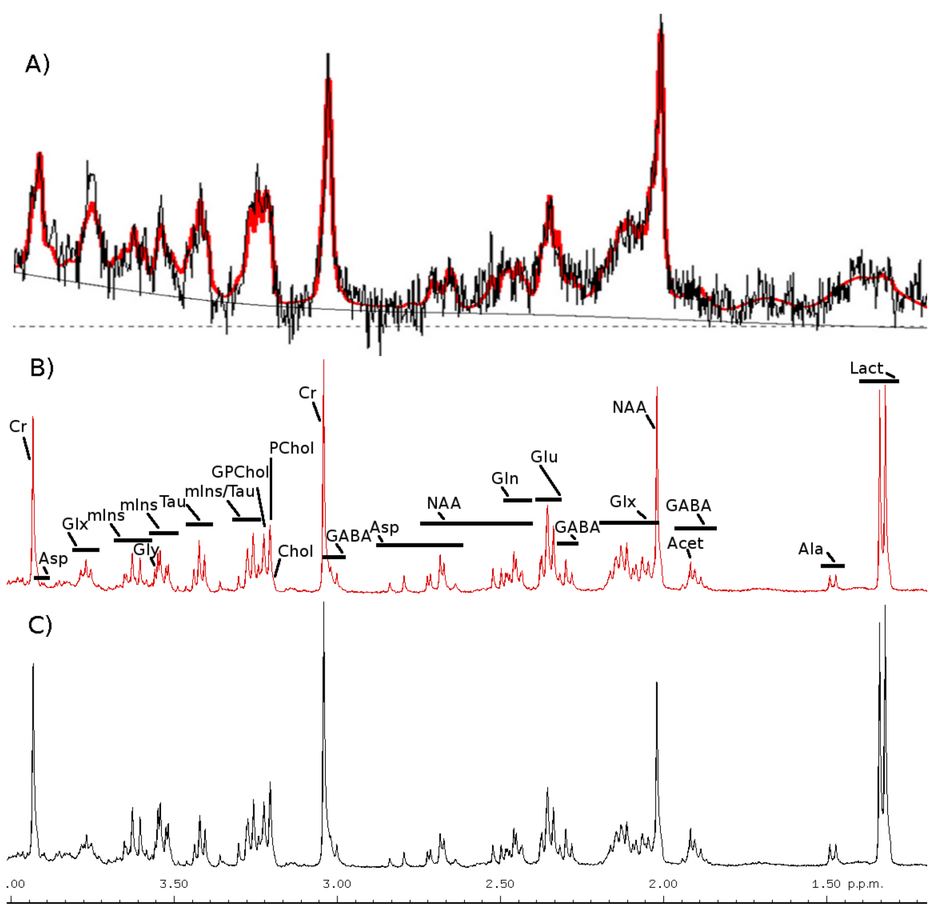

Fig 1. ***In Vivo*** [A)] **and** ***In Vitro*** [B)/C); methanol extracts] **^{1}H NMR Spectra of the Frontal Cortex** [A)/B)] **and Hippocampus** [C)]. *In vitro* spectra were acquired at 400 Mhz and *in vivo* at 300 MHz.

In Vivo – *In Vitro* and Brain Areas.

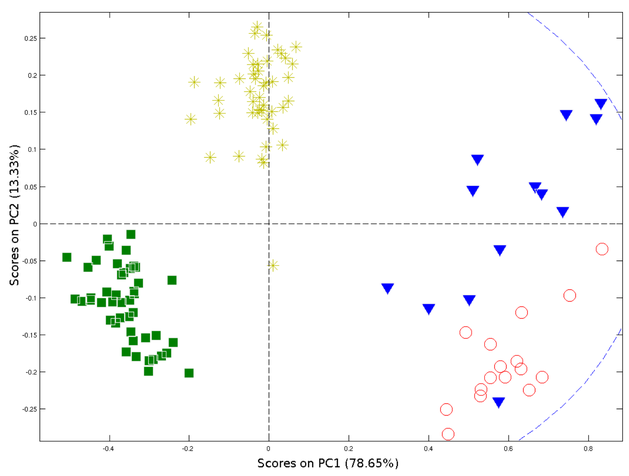

Fig 2. PCA Scores Plot of All Samples Analysed, classed according to marker: dark blue triangle – *in vivo* frontal cortex, dark yellow star – *in vitro* frontal cortex, red circle – *in vivo* hippocampus, green square – *in vitro* hippocampus.

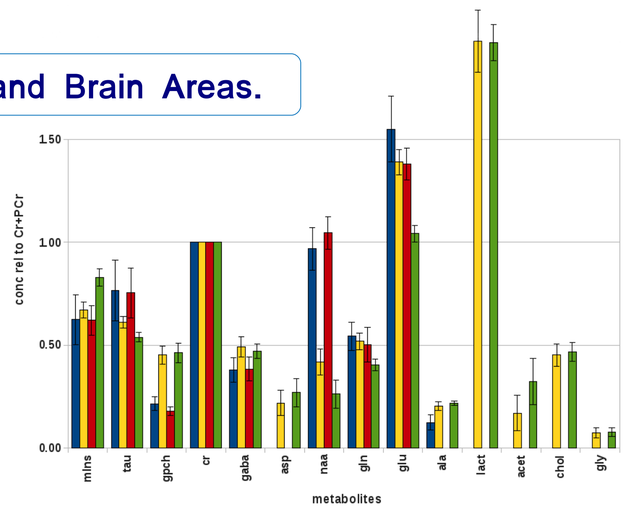

Fig 3. Column Chart of All Relative Signal Intensities Analysed, classed according to colour: blue – *in vivo* frontal cortex, dark yellow – *in vitro* frontal cortex, red – *in vivo* hippocampus, green – *in vitro* hippocampus.

All *in vivo* and *in vitro* intensities were significantly ($p<0.05$) different in the respective brain areas, except mIns and Gln in the frontal cortex.

Intensities that significantly differed between *in vivo* brain areas were (G)PChol and Glu; *in vitro* intensities all differed between the frontal cortex and hippocampus, except (G)PChol, GABA, Lact, Chol and Gly.

Social Isolation.

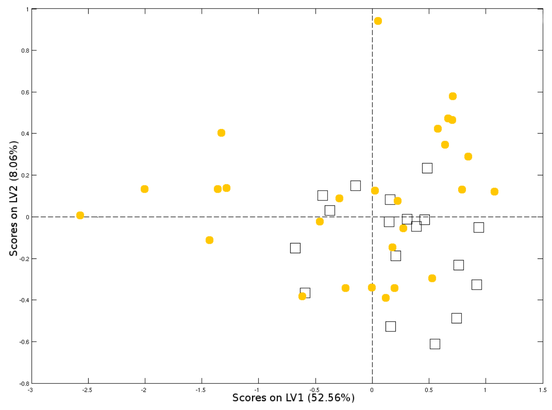

Fig 4. PLS-DA Scores Plot Indicating No Metabolic Effect of Post-weaning Social Isolation in the *in vitro* Frontal Cortex, classed according to marker: black box – isolation-reared, yellow circle – group-housed; for 3 LVs: $r^2x(cum)=60.61\%$, $r^2y(cum)=17.64\%$.

Conditions During *In Vitro* Spectroscopy.

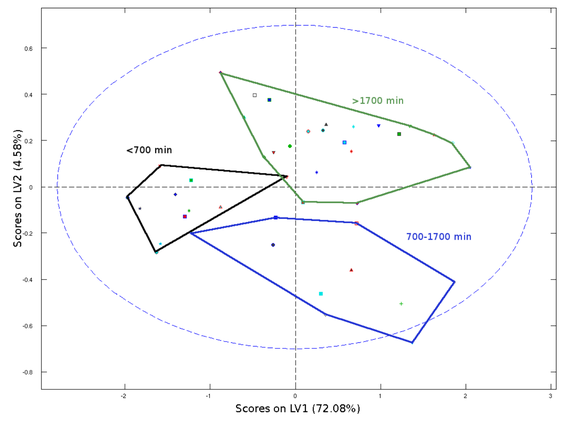

Fig 5. PLS Scores Plot Suggesting a Metabolic Effect of the time span during which Hippocampus Extracts were subjected to Room Temperature Prior NMR Spectroscopic Analysis (≤41 hours)**,** classed according to colours: black – <12 hours, blue – 12–28 hours, green – >28 hours. for 3 LVs: $r^2x(cum)=79.74\%$, $r^2y(cum)=80.73\%$, R^2(prediction by cross-val.)=0.664.

DISCUSSION.

***IN VIVO* AND *IN VITRO* RESULTS AGREED IN BIOLOGICAL QUESTIONS:**

No metabolic difference between animals reared in isolation and groups; in agreement with a previous report suggesting no basal alteration of NAA in the frontal cortex or hippocampus after social isolation (Fone and Porkess, 2008).

Metabolite patterns differed between the frontal cortex and hippocampus; in accordance with an earlier publication (Salek et al., 2008).

***IN VIVO* AND *IN VITRO* RESULTS DIFFERED IN:**

Relative metabolite abundance; disagreeing with Barker et al. (1995), but agreeing with Petroff et al. (1995) and Tracey et al. (1996).

Coefficients of Variation; better spectral resolution in and greater number of *in vitro* spectra enabled more precise concentration calculations, except for NAA.

CONDITIONS DURING SPECTROSCOPIC MEASUREMENTS ALTERED METABOLIC PROFILES IN METHANOL EXTRACTS:

Subjection to **room temperature for up to 41 hours** changed levels of Acet and Asp (increasing), and NAA and probably Lact (decreasing); [likely process: NAA→Acet+Asp].

FACTORS THAT DID NOT AFFECT RELATIVE METABOLITE LEVELS IN METHANOL EXTRACTS: single-dose **memantine injection** (1 week prior sacrificing), **family** affiliation, **tissue storage** (–80°C, up to 6 months), **tissue mass** and **body mass** – not shown.

CONCLUSION.

Results obtained from *in vivo* and *in vitro* MR spectroscopy were different in many aspects, but agreed in biological research questions.

Post-mortem effects might have occurred in methanol extracts of brain tissue thereby causing unwanted metabolite concentration alterations.

For future studies, care must be taken when *in vitro* data is tried to be translated into living organisms.

REFERENCES.

Barker et al. (1994) *Magnetic Resonance in Medicine,* 32(2):157–163.
Fone and Porkess (2008) *Neuroscience & Biobehavioral Reviews,* 32(6):1087–1102.
Petroff et al. (1995) *Magnetic Resonance Imaging,* 13(8):1197–1211.
Salek et al. (2008) *Neurochemical Research,* 33(2):292–300.
Tracey et al. (1996) *Journal of the Neurological Sciences,* 141(1-2):13–18.
Wu et al. (2008) *Analytical Biochemistry,* 372(2):204–212.

ACKNOWLEDGEMENTS.

I am grateful to my supervisors **Prof. Dorothee P. Auer** and **Dr. Clare A. Daykin** and their respective schools, *i.e.* **School of Clinical Sciences** and **School of Pharmacy**, for their support; and to **Prof. Kevin Fone** and **Dr. Marie-Christine Pardon** for their collaboration.
Many thanks to **Dr. Florian Wuelfert** and my **lab and office colleagues** for their time and patience;
and to **Bsc Hons Neuroscience Julia Long**, **Mmag. Marija Dabic**, **Mag.pharm. Monique Huber** and **Dr. Patrik Muigg** for their wise words.