Assessment of Stress-induced Neurochemical Alterations in a Rat Model of Chronic Stress using *in vivo* ^{1}H MRS at 11.7 Tesla

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Target Audience Scientists interested in the application of MRS to study psychiatric disorders in particular stress-induced neurochemical alterations.

Purpose As demonstrated in previous studies, stress induces anatomical, functional and cellular changes in specific neural circuits particularly the hypothalamus-pituitary-adrenal (HPA) axis[1-4]. In this study, stress-induced neurochemical alterations were explored in a rat model of chronic stress using *in vivo* NMR Spectroscopy (MRS) at 11.7 T; concomitantly potential correlations with the plasma level of corticosterone ([Cort]) as a biomarker of stress response were investigated.

Stress Paradigm 30 male, 9 week-old Wistar rats were submitted 1h per day to the « Chronic Unpredictable Stress » ("CUS") paradigm[5] for 3 weeks. Briefly, animals were exposed once daily to one of several aversive stimuli: cold water (18°C), vibration, restraint, overcrowding or exposure to a hot air stream. The stressors were presented in random order for the duration of the experiment. This stress paradigm was shown previously to result in elevated [Cort]. Another group of aged-matched 15 control rats ("Ctrl") were handled daily and served as controls. Corticosterone levels were measured in blood serum sampled between 8:00 and 9:00 A.M. after the stress protocol using a commercially available ELISA kit.

MRS Acquisitions MRS data were collected from all rats (then 12 week-old). Animals were anesthetized using isoflurane (1-2%). Body temperature was monitored and maintained at 37° ± 0.5°. All Experiments were performed on a 11.7 T/16 cm Bruker BioSpec MRI scanner using a 72mm volumic resonator for transmission and a 4-channels surface coil for reception. Anatomical images were acquired for positioning using a TSE sequence. ^{1}H MR Spectra were acquired with a LASER[6] sequence (TE/TR=25/3500ms, 128 averages, 2048 points) from a 18 µL volume encompassing the right dorsal hippocampus (Figure 1). B_0 shimming was performed using the B_0 mapping and localized shim Bruker routines (water linewidth = 13 ± 1 Hz). Water suppression was done using VAPOR[7].

Data Analysis A total of 42 MR spectra (28 CUS and 14 Ctrl) were analyzed using LCModel[8] and a set of simulated spectra. The signal of macromolecules (MM) was parameterized as described elsewhere[9] and implemented in LCModel. Metabolite concentrations were derived using the total Creatine (tCr) signal as an internal reference of concentration ([tCr]=8mmol/L). Statistical significance between groups was established using a bilateral t-test.

Results and Discussion Figure 1 shows a ^{1}H MR spectrum, the voxel localization as well as the contributions of the metabolites of interest. As illustrated by the box-plots in Figure 2, the chronic stress led to an increase in GABA (+14%, p<0.05) accompanied with minor decreases in NAA (-3%, p<0.05) and MM1 (-6%, p<0.05). When considering [Cort] to discriminate within each groups (see Fig 2 for sub-groups definition), one can notice a net difference in Glu/Gln ratios between CUS rats with low and high [Cort] (H CUS vs L CUS: +27%, p<0.01 ; similar to H CUS vs L Ctrl: +31%, p<0.01). Interestingly, the normalized Glu/Gln ratio in "L CUS" rats was accompanied by a moderate increase in Ins level (L CUS vs H CUS: +7%, p<0.05).

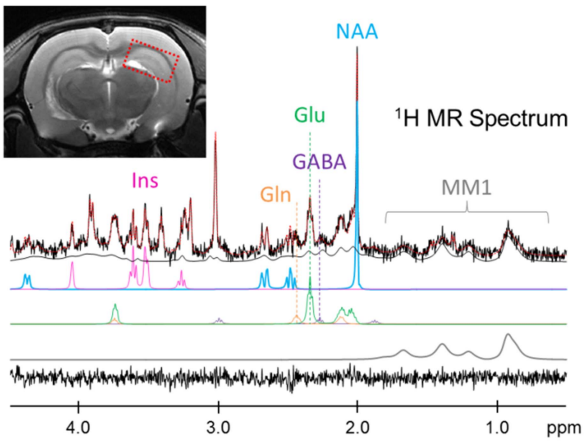

Fig.1 Neurochemical profile acquired from a VOI (3.5x2.5x2 mm^3) encompassing the dorsal right hippocampus. The LCModel fit and the spectral contributions of the metabolites of interest are shown.

Conclusion Our observations are consistent with a moderate neuronal metabolic stress and the up-regulation of GABAergic neurotransmission to limit the HPA axis hyper-activation due to our 3 week-long chronic stress paradigm. Notably, resilience to longitudinal stress (i.e. low [Cort]) in CUS rats is associated to a likely "neuroprotective" glial activity.

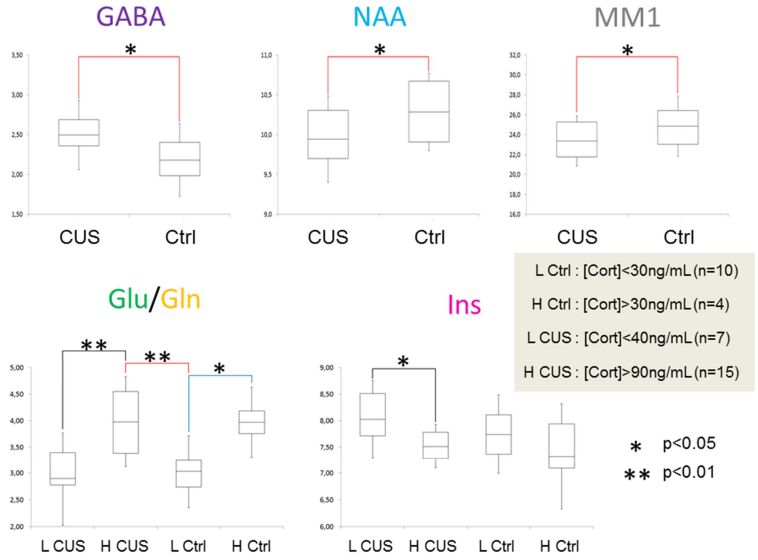

Fig.2 Box-plots of GABA, NAA and MM1 concentrations (in mmol/L) in CUS (n=28) vs Ctrl (n=14) rats and box-plots of Glu/Gln and Ins for the sub-groups: L CUS ([Cort]<40ng/mL, n=7), H CUS ([Cort]>90ng/mL, n=15), L Ctrl ([Cort]<30ng/mL, n=10) and H Ctrl ([Cort]>30ng/mL, n=4). Statistical significance is indicated using * p<0.05 and ** p<0.01. 1st and 3rd quartiles define each box, the central bar being the mean values and error bars corresponding to the SDs.

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