Neurochemical profile of the rat lateral septum investigated with 1H-MRS

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Introduction: A large number of studies have examined the consequences of exposure to stress on aggressive behavior both in rodents (1, 2). In order to investigate the potential neurobiological mechanisms involved in stress-induced aggression, the lateral septum (LS) is often chosen since this brain area has been shown to be involved in the expression of anxiety-behaviors such as those involved in conflict procedures or exploration tests (2). Despite the aforementioned interest, the neurochemical profile of LS has never been analyzed to our knowledge with high-field MRS techniques. The aim of the present study was therefore to measure and characterize the LS metabolic profile using proton MRS.

Materials and Methods: **Animals:** Each male Wistar rat (n=17, 495 ± 56 g) was anesthetized using isoflurane (2-3% in O_2) and was placed in a dedicated stereotactic rat holder under continuous isoflurane anesthesia (2% in O_2). The rat body temperature was monitored continuously by a rectal probe and maintained at a physiological temperature (37°±1°C) using warm circulating water around the animal. **1H-MRS** All the experiments were performed on an actively shielded 9.4T/31cm bore magnet (Magnex, Varian) with 12cm gradients (400mT/m in 120µs). A quadrature Transmit/Receive 17mm surface coil was used. First and second order shims were adjusted using FASTMAP (3) in a 27-45µl volume placed over the lateral septum by reference to the Paxinos atlas (4) and using high –resolution multi-slice fast spin echo (FSEMS) images. Localized Proton spectroscopy was performed using SPECIAL (5). 40 blocks of 16 FIDs were acquired for a total acquisition time of 40 minutes. Metabolite concentrations were calculated using LCmodel (6). An unpaired t-test was used to compare with metabolites obtained in a voxel including frontal cortex, corpus callosum and striatum in the rat brain at 9.4T in (5), A p value<0.05 was considered significant.

Results In Fig1, the multi-slice FSEMS images show the voxel position for proton MRS measurements in the lateral septum. Under isoflurane anesthesia, shimming this area of the rat brain was easy with a mean water linewidths of 13±3Hz. In Fig2, a typical single voxel spectrum of the lateral septum is shown with peak assignments. Fig.3 compares the mean metabolite concentrations (±standard deviations) calculated in the lateral septum with the mean metabolite concentrations (±standard deviations) obtained in an unspecific brain region in (5). Significant changes were obtained for Asc ($p<0.05$), GPC ($p<0.01$), PCr ($p< 0.05$), and Glu ($p<0.0001$).Some tendencies were also observed but not significant ($p>0.05$) with lower concentration estimates in LS for Asp, Tau, PE but higher estimated for myo-Ins, Gln, and Glc. The mean Cramer-Rao lower bounds (CRLB) (Table1) were all under 30% but however higher in LS than for the brain data at 9.4T.

Discussion/Conclusion: In the present study, the neurochemical profile of the rat lateral septum was measured and characterized at 9.4T and demonstrated statistically significant changes compared to the neurochemical profile measured in an area of the brain encompassing both cortical and striatal tissues. The lateral septum is an interesting area to further investigate cognitive and metabolic changes in relation to stress using high field MRS techniques. The accurate and specific neurochemical characterization of the LS will be useful to further understand changes related to stress exposure.

References(1) Duncan G et al. Brain Res, 1265, 186-195, 2009 (2) Stemmelin J et al. Neuropsychopharm., 30,35-42, 2005 (3) Gruetter R et al. MRM,29:804,1993 (4) Paxinos G, 1985 Academic press (5) Mlynarik V et al. MRM, 56:965,2006 (6) Provencher SW.MRM,30:672,1993

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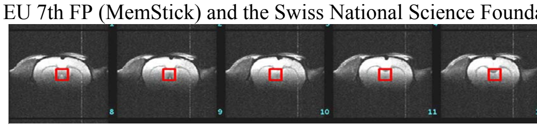

Figure1: Fast Spin echo images (TR/TE= 2500/52ms; AVE=8; FOV=18x18mm; TH=0.5mm; 256x256; Acq=8minutes) showing a voxel positioned over the lateral septum in the rat brain

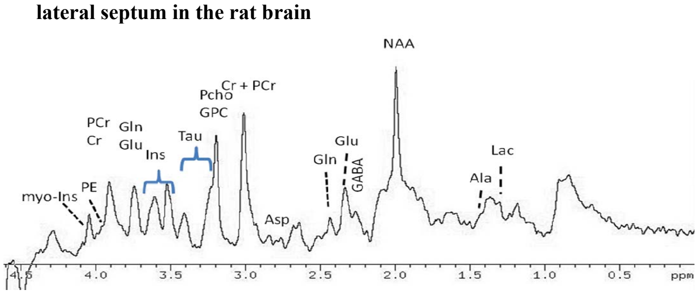

Figure2: Example of proton 9.4T spectrum from the rat lateral septum with peak assignments.

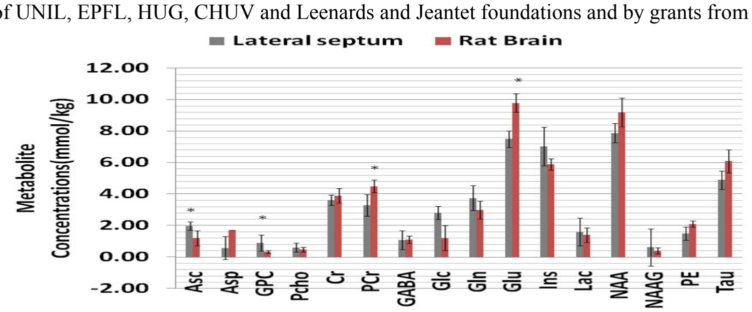

Figure 3; Mean metabolite concentrations (±Standard deviations) in the LS and in an unspecific brain area in (5)

Metabolite	LS CRLB(%)	Brain CRLB(%)
Asc	15.1	14
Asp	36	14
GPC	21.4	18
Pcho	20.8	16
Cr	6	4
PCr	5.6	3
GABA	11.8	9
Glc	23.8	30
Gln	4.9	3
Glu	3.05	1.5
Ins	2.9	2
Lac	11.7	7
NAA	1.875	1
NAAG	14.4	6
PE	11.3	6
Tau	4	2

Table 1: Mean Cramer-Rao Lower Bounds (CRLB) obtained in LS and in the rat brain in (5)