Brain Neurochemical effects of long-term sleep fragmentation investigated in mice at 14.1T using 1H-MRS

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Introduction: Sleep-fragmentation (SF) is a common symptom associated to disturbances such as sleep apneas (1) Previous studies showed that SF induces daytime sleepiness and cognitive impairments associated to metabolic changes (2, 3). In the present study, the neurochemical profile of mice submitted to SF during 14 days was examined using ^{1}H-MRS at 14.1T both in hippocampus and the frontal cortex. The goal of this study was to investigate changes linking sleep fragmentation to modifications in neurotransmitter concentrations.

Materials and Methods: Animal preparation: C57BL6 mice (n=10, BW=25±2g) were individually transferred in rotating Plexiglas cylindrical cages equipped with three internal Plexiglas static walls to be submitted to SF. The rotations consisted in a mild rotation (1.5 rpm) of the floor of the cages lasting 20s every minute. Such a rotation brings the mouse in contact with one of the walls and wakes it up. Animals had free access to food and water. Each mouse was left to adapt to its new environment for a week before the first MRS session and prior to the start of the cage rotation to prevent stress. Mice underwent a first MRS session in their basal state and a second MRS 14days after .Prior to MRS, mice were gently manipulated to avoid stress and anesthetized with 2% isoflurane in a mixture of O_2 and N_2O. Each of them was positioned in a dedicated mouse holder equipped with ear and teeth bars and anesthetized with 1% isoflurane in O_2/N_2O through a nose cone. Body temperature was maintained at 36.5±1ºC by circulating warm water around the animals.**^{1}H-MRS** All the experiments were performed on a 14.1T/26cm horizontal bore magnet (Magnex, Varian). A quadrature T/R 14-mm surface coil was used. Localized proton spectroscopy was performed using SPECIAL (4) in a 4±1µl voxel for the hippocampus and in a 12±1µl voxel for the frontal cortex placed using multi-slice fast spin echo (FSEMS) images. Field homogeneities were adjusted using FASTMAP (5) up to water linewidth=20-25Hz. At least 40 blocks of 16 FIDs were acquired for a total acquisition time of 40minutes. Metabolite concentrations were calculated using LCmodel (6). A paired t-test was used to compare pre and post-SF metabolite concentrations. A pvalue<0.05 was considered significant.

Fig1a-1b: Comparison of mean metabolite concentrations (mean±SEM) in the hippocampus(1a) and the cortex(1b) between basal and SF states.

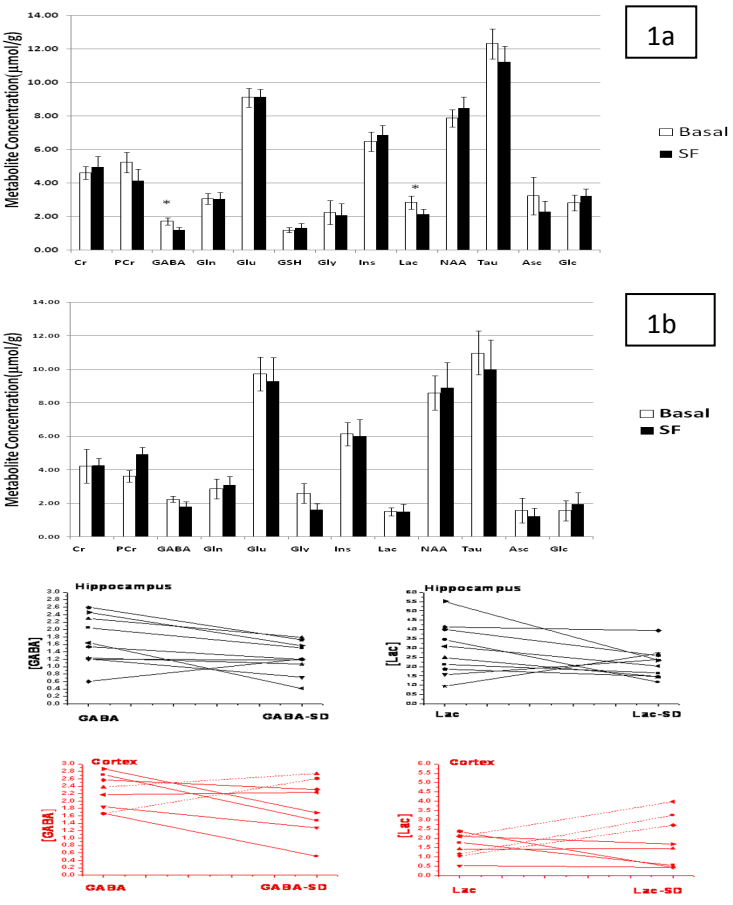

Fig2: Individual changes of GABA and Lactate in hippocampus (black) and cortex (red) due to sleep fragmentation.

Results: Fig.1a and 1b present the comparison of mean metabolite concentrations pre and post-SF in the hippocampus and the frontal cortex respectively. In the hippocampus, mean GABA concentrations decreased significantly between basal and SF states (pvalue =0.006) and a significant decrease was also detected for lactate (pvalue<0.05). In the cortex, no significant differences were observed between basal and SF states, however a tendency to decrease was also observed for GABA (pvalue=0.21). In Fig.2, the evolution of GABA, and Lactate concentrations between basal and SF states are shown individually in the hippocampus (Black) and in the cortex (red).

Discussion and Conclusion: Sleep fragmentation produces cognitive deficits both in humans and rodents. Spatial memory is dramatically affected in rodents after SF (2). The hippocampus plays a key role in the processus of spatial learning. Here, we report significant decreases in GABA and Lactate in hippocampus after long-term SF. Lac decrease may reflect a decreased synaptic function in the hippocampus since it is a favorite energy supplier for activated neurons (7). GABA is involved in the production of the theta rythm of the hippocampus. Hence, the decrease in GABA may contribute to poor hippocampal functioning.

References:(1)Stepanski, Sleep, 25:268-276,2002 (2) Tartar et al, EJN, 23:2739-2748, 2006 (3)Mc Kenna et al. Neurosc,146:1462-1463, 2007 (4) Mlynarik V et al. MRM, 56:965,2006 (5)Gruetter R et al. MRM,29:804,1993;(6) Provencher SW.MRM,30:672,1993;(7) Magistretti et al., Science,283:496-7,1999

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