Hippocampal alterations in congenital learned helpless rats after electroconvulsive seizures detected with in vivo 1H MR-Spectroscopy at 9.4T

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Introduction

Depression is the second most frequent disease in the western world. Nevertheless there is a big lack in pharmacotherapy as one third of the patients do not respond to pharmacological therapy after two months. Electroconvulsive therapy (ECT) has a fast initiating antidepressive effect. We investigated a well validated animal model for treatment resistant depression, namely the congenital learned helplessness (cLH) model in rats with high field proton magnetic resonance spectroscopy (^{1}H-MRS) at 9.4 T following a 6 days electroconvulsive shock (ECS) treatment. The high spectroscopic resolution of the scanner allows quantifying separately choline, taurine, glutamate and glutamine. We hypothesized that the fast initiating antidepressive effect of electroconvulsive therapy is associated with an increase of glutamate and choline, indirectly reflecting synaptogenesis and glutamatergic activity in the hippocampus [1].

Learned helpless rats treated with ECS

Animals exposed to inescapable foot shocks develop in approximately 20% helpless behavior. These rats can be identified by exposing them to the shock 24 h after the training when they fail to learn the escape.

A breeding program using Sprague Dawley rats was established in which rats were selected on the basis of their susceptibility to develop learned helplessness (LH). They were mated for subsequent generations. In the 29th generation a strain (cLH), which exhibits helpless behaviour without preshock, emerged. [2]

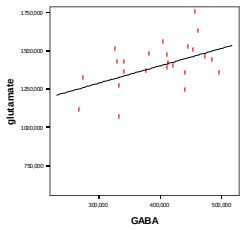
In our study we used 12 cLH rats aged 14 weeks. Six of the helpless rats were treated with electroconvulsive seizures (ECS) for six days the other two groups rested as control groups. Helpless behavior was once tested before the treatment started and a second time at the morning of the ^{1}HMRS.

Quantification of brain metabolites with 1H MRS

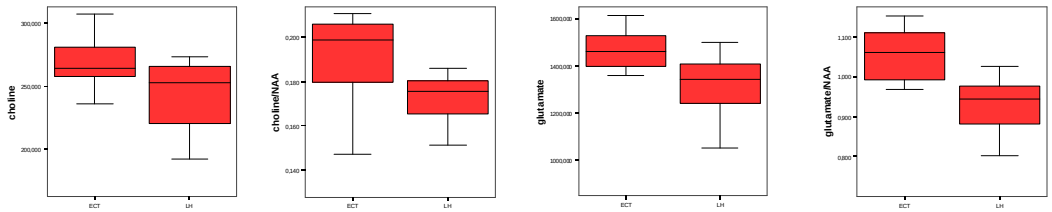
Both hippocampi of all rats were investigated with in vivo single voxel ^{1}H MRS in a 9.4T animal MR scanner (Bruker, Rheinstetten, Germany) under anesthesia with 2.3% isoflurane in a 50%/50% mixture of O_2 and air. Vital signs where monitored throughout the experiment. Voxel from left and right hippocampi were acquired from each animal using a PRESS single voxel sequence with total echo time = 10ms, total repetition time = 4s and 256 acquisitions resulting in a total acquisition time of 17 min. The voxel were positioned and angulated in the left and right hippocampal area. For optimal volume coverage and signal/noise-ratio a voxel size of 2x2x4 mm³ was chosen. To ensure good B_o homogeneity 1st and 2nd order shimming was conducted with fastmap over a volume of 4x4x4 mm³. Postprocessing and quantification of spectra were done with LCModel [] using phantom basis data sets including 22 metabolites measured with the same scanner. The brain extraction and brain segmentation were achieved by FSL Brain Extraction Tool (BET) and FSL Automated Segmentation Tool (FAST) respectively. In the voxel geometry, there were about 4000 sample points, which were segmented as different tissue types. The amount of the tissue types was estimated by the segmentation result of the sample points in statistics [3].

Results

After a six day course of ECS no behavioral changes could be observed in the LH test which would have been to assume after four weeks of ECS [5]. We could not find alterations in contents of grey and white matter after ECS in the segmented brain images. The rats which had received ECS showed a significant increase in tCho/NAA (tCho=glycerophosphocholine + phosphocholine, NAA=N-Acetylaspartate) (p=0.007), which could be corroborated with a significant increase of absolute choline concentration. These results are in concordance to previous results at 4.7 T [4]. The main finding of the study is a significant increase of absolute glutamate (p=0.003) after ECS, which is supported by asignificant increase of glutamate/NAA (p=0.000). The GABA concentration increased similarly. Interestingly, the increase of glutamate significantly correlated with an increase of y-aminobutyric acid (GABA) (R=0.504, p=0.12). Additionally, the glutamine concentration was unaltered by ECS, as well as the ratio of Glu/GABA



Correlation of glutamate to GABA

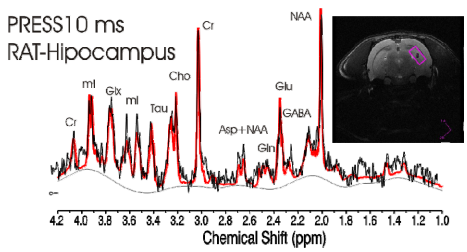


Boxplots of choline, choline/NAA, glumate and glutamate/NAA in cLH having received ECS (ECS) and naïve cLH (LH)

Discussion

With the help of segmentation and water scaling we could corroborate our first preliminary results from metabolites ratios to NAA.

We could replicate the finding of an increase of choline after ECS [2,5], which corresponds to a hypothesized ECT evoked synaptogenesis. Glutamate, which increases significantly, is the most important neurotransmitter in the brain. It is known to play an important role in the pathophysiology of depression [6]. As ECS increases glutamate without affecting glutamine this could indicate an intervention in the glutamate-glutamine cycle where both metabolites are strongly connected to each other.



Example of voxel positioning and example Spectrum

References:

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