MRI and MRS characterization of Crtc1 knock-out mice limbic structures: investigating neurobiology of mood disorders

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Introduction:

In vivo MRI and MRS have been two extensively used non-invasive techniques for characterizing psychiatric disorders. However, findings in humans show discrepancies between the studies, which reflect the lack of understanding in the pathophysiology of these complex diseases and reinforces the need for a strengthened endophenotypic characterization in order to make an accurate diagnostic. Mouse models have become promising tools for studying psychiatric diseases since they allow linking invasive molecular and behavioral experimentation with techniques applicable to humans like MRI and MRS. We have investigated the metabolic and volumetric status of a previously reported mouse model of mood disorders lacking an important brain plasticity gene, *Crtc1* (CREB-regulated transcriptional coactivator 1). *Crtc1* knock-out animals are considered as relevant for studying mood disorders since they show neurobehavioral depressive-like endophenotypes as well as late-onset obesity together with monoaminergic system dysfunctions.[1,2]

Method:

All animal studies were performed with the approval of the local animal care and use committee. *Crtc1*$^{-/-}$ animals[1] (KO, n=12) and their countertypes (WT, n=7) at the age of 6 weeks were scanned in a horizontal 14.1 T magnet, under isoflurane anesthesia (1-2%).[1] Briefly, the animals were fixed in holder with a bite piece and two ear bars and anatomical MR images were obtained using T_2-weighted FSE images (25×0.6mm slices, TE_{eff} /TR=50/4000ms, nt=8). This set of images was used for volumetric measurements and also localizing the volumes of interests (VOIs), including dorsal hippocampus (1µl), prefrontal cortex (PFC, 2.7µl), ventral hippocampus (3.1µl) and amygdala (AM, 1.9µl). After adjusting field inhomogeneity (water linewidth <20Hz) on the target VOI, localized ^{1}H MRS was applied using SPECIAL sequence (TE/TR=2.8/4000ms)[3]. In order to reach satisfactory SNR, i.e. >6, from each VOI, the number of scans were in the range of 240-800.

The spectral data were frequency corrected and summed for further quantification using LCMODEL (Mlynarik et al.,[3] and reference therein) referencing the endogenous water (80% in brain tissue) from the identical VOI. Cramér-Rao lower bounds (CRLB) > 50% were considered not reliable.

A pattern-based protocol was used to determine the main structural changes of ventricles, hippocampus and prefrontal cortex.

Results:

Anatomical images were obtained with satisfactory quality to identify the VOIs. *In-vivo* spectra from prefrontal cortex, dorsal and ventral hippocampus as well as amygdala were obtained in excellent quality (Figure 1), with resulting metabolic LWHMs of 13±3 Hz, 10±3 Hz, 12±3Hz and 14±4 Hz and with SNRs of 11±3, 8±2, 11±3 and 7±2, respectively. Spectral characteristics of these four regions were visually observed in taurine, myo-inositol and lactate etc. Quantification of the spectral data confirmed the visual observation (two way ANOVA p<0.05).

The similar quality of both MRI and MRS results were obtained in the KO mice.

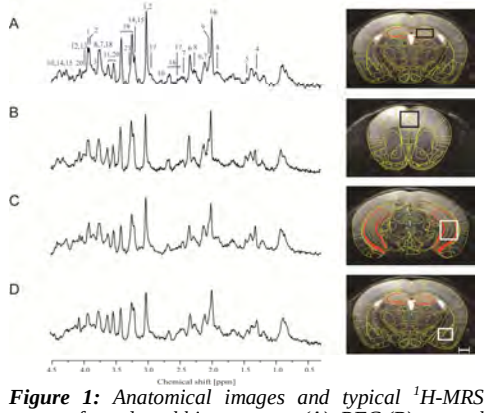

Figure 1: *Anatomical images and typical ^{1}H-MRS spectra from dorsal hippocampus (A), PFC (B), ventral hippocampus (C) and amygdala (D) of WT mice.*

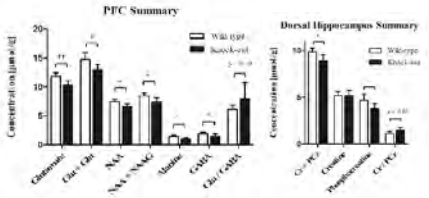

Figure 2: *Summary of significant metabolic changes relating to GABA/Glutamatergic system and energy metabolism in PFC and dorsal hippocampus. Significant levels were indicated by "*" for p<0.05 and "**" for p<0.005. Abbreviations: Glu, glutamate; Gln, glutamine; NAA, N-acetylaspartate; NAAG, N-acetylaspartylglutamate; GABA, γ–ammino-butyric acid; Cr, Creatine; PCr, Phosphocreatine.*

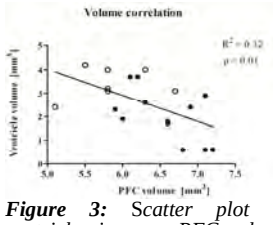

Figure 3: *Scatter plot of ventricle size over PFC volume between WT (open circles) and KO mice (solid circles). A Pearson correlation line was drawn over the entire data set.*

The region-specific characteristic in both spectral pattern and metabolic profile was also observed. When comparing to the WT animals, a significant number of changes were found mainly in the PFC and the dorsal hippocampus (Figure 2). For instance in the KO mice, glutamate and GABA were reduced in PFC, whereas a marked reduction of the energy metabolite phosphocreatine was visible in the dorsal hippocampus.

Volumetric analysis of the MR images revealed a significant correlation (p=0.01) between shrinkage of ventricles and swelling of surrounding tissue in the KO mice (Figure 3).

Discussion:

To our knowledge, this is the first report of ^{1}H MRS measurement in murine amygdala and of studying four limbic structures in *Crtc1*$^{-/-}$ mice mimicking mood disorders. *Crtc1*$^{-/-}$ mice present a strong reduction of ventricular size correlating with an increase of adjacent gray matter, and the energy- and glutamate/GABA metabolism shows dysfunction with a high region-specificity. Such strong metabolic and volumetric alterations in the *Crtc1*$^{-/-}$ mice are similar to human findings in mood disorders[4,5], which suggested that GABA/glutamatergic system or the energy metabolism are impaired in some specific regions of the brain.

Conclusion:

Here we provide the link between a specific gene and its associated metabolic and volumetric alterations, which will help understanding further the underlying pathophysiology of mood disorders.

References: 1. Breuillaud, et al. Biol Psychiatry. 2012; 72(7):528-36. **2.** Breuillaud, et al. Nature Medicine. 2009; 15(9) :989 -990. **3.** Mlynarik, et al. Magn. Res. Med. 2006; 56:965-970. **4.** Sanacora, et al. Neuropharmacology. 2012; 62(1):63-77. **5.** Stork C and Renshaw PF. Mol Psychiatry. 2005;10(10):900-19

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