Detection of structural and functional changes in the cortex of Bassoon-mutant mice by MRI and 1H-NMR-Spectroscopy

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Introduction

Neurotransmitter release is a strictly controlled membrane trafficking process at specific sites of the presynaptic plasma membrane, the so-called active zones. At the ultra-structural level the active zone appears as an electron-dense region beneath the presynaptic membrane, which biochemically corresponds to a network of cytoskeletal and associated proteins: the cytomatrix at the active zone (CAZ). To study the role of one component of the CAZ, the *Bassoon* protein in the organization of various steps of the synaptic vesicle cycle, a mouse mutant lacking the central region of the protein was generated (1). Bassoon-mutant mice are characterized on a cellular level by a reduction in normal synaptic transmission in a subset of neurons. To elucidate the consequence of such a malfunction on a systemic level we employed manganese-enhanced magnetic resonance imaging (ME-MRI) and *in vitro* high-field (14.1T) ^{1}NMR-Spectroscopy as a screening tool to compare Bassoon-mutant mice with wild-type littermates.

Methods

Mice lacking a functional Bassoon (BsnΔEx4/5) were generated as described previously (1). *MR imaging*: Mice were anesthetized with 1.0-1.5 % isoflurane (in 70:30 N_2O:O_2; v:v). MRI experiments were performed on a Bruker Biospec 47/20 scanner at 4.7T equipped with a BGA 12 (200 mT/m) gradient system and a 25 mm Litzcage small animal imaging system (DotyScientific Inc., Colombus, SC, USA). Two days before MRI measurement, animals were injected subcutaneously with an aqueous solution containing 1 µmol/g $MnCl_2$. A 3D data set of T_1-weighted images was obtained using a MDEFT pulse sequence with the following parameters: TR 21.18 ms, TE 4.00 ms, flip angle 15º, FOV 30x30x20 mm, matrix 256x256. *^{1}H-NMR-Spectroscopy* For high-field ^{1}H-MRS, brains were separated into cortex, cerebellum, and hippocampus. Metabolites were extracted with 5% perchloric acid. ^{1}H-MR spectra were acquired on a Bruker DRX 600 spectrometer with cryo-platform (Bruker Biospin GmbH, Karlsruhe, Germany) operating at 14.1 T. The following parameters were used: 9.5 µs pulse length (90° pulse), 7,200 Hz sweep width, digital resolution of 32k data points, repetition time 10 s, saturation of the residual water signal by a frequency-selective pulse before each free induction decay

Results & Conclusion

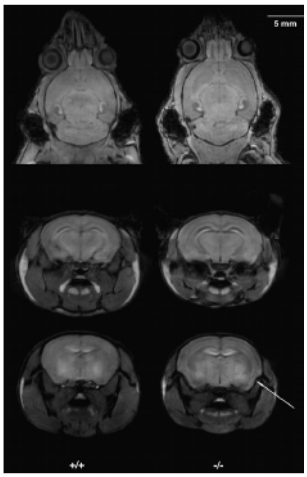

***Fig. 1**. Comparison of manganese uptake into brain structures of wild-type and Bassoon-mutant mice. ME-MRI was used to detect differences in the manganese distribution within the brain. The main difference in Mn^{2+} uptake was found within the cortex. Both, in horizontal sections (upper row) and frontal sections (lower 2 rows) the cortex appears more laminated, pointing to a cortex layer dependent variation in total manganese uptake.*

Main findings of this study are: (I) Absence of a functional presynaptic protein Bassoon in mice causes an enlarged brain size, which is mainly caused by an increased cortex and hippocampus volume. (II) The observed increase in cortex size in Bassoon-mutant mice is paralleled by an altered manganese uptake and accumulation within cortical layers. (III) The increase in cortex thickness and altered manganese uptake is not associated with obvious changes in cortical lamination. (IV) The basal neuronal activation pattern within the cortex of Bassoon-mutant mice differs from wild-type mice. (V) Alterations in cortical functions are paralleled with metabolic perturbations as revealed by high resolution (600 MHz) ^{1}H NMR spectroscopy. (VI) The concentrations of N-acetylaspartate (NAA, a neuron-specific metabolite), glutamate, and glutamine are significantly reduced in the cortex of Bassoon-mutant mice. (VII) Coinciding with the reduction of NAA, the neuronal density is reduced especially in cortical layer IV of Bassoon-mutant mice. Consequently, the combination of MRI and ^{1}H-NMR spectroscopy revealed that a minor malfunction in synaptic transmission affects on a systemic level the cortical architecture as well as the basal cortical activity in Bassoon-mutant mice. Furthermore, this study exemplified that MRI/NMR-spectroscopy are helpful tools to characterize genetically modified mice.

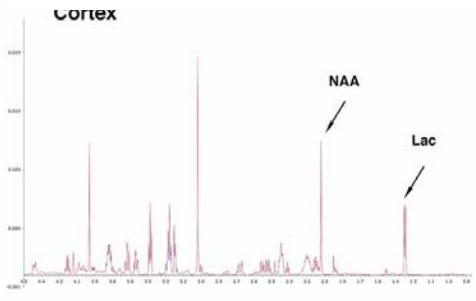

***Fig. 2**. Comparison of high resolution 600 MHz ^{1}H-NMR spectra of an aqueous extract of cortex tissue taken from wild-type (blue line) and Bassoon-mutant mice (red line). Shown are the averages from each 8 wild-type and 8 Bassoon-mutant mice. The difference in the concentration of NAA was significant.*