Early life stress impairs serotonergic neurotransmission specifically in the prefrontal cortex revealed by pharmacological fMRI in mice

F. Razoux*[1], H. Russig*[2], T. Mueggler[1], C. Baltes[1], I. M. Mansuy[2], and M. Rudin[1,3]

[1]Institute for Biomedical Engineering, University & ETH Zurich, Zurich, Switzerland, [2]Brain Research Institute, University & ETH Zurich, Zurich, Switzerland, [3]Institute of Pharmacology & Toxicology, University Zurich, Zurich, Switzerland

INTRODUCTION: Early life stress constitutes a risk factor for the development of depression in human[1]. Early life stress was modeled in C57Bl6 mice using unpredictable maternal separation combined with unpredictable maternal stress (MSUS). These mice tested as adults, exhibited a depression-like phenotype in the Porsolt swim test. To investigate the role of the 5-HT1A receptor, known to be strongly implicated in the pathophysiology of depression [2], we established a functional magnetic resonance imaging (fMRI) protocol for mice using the 5-HT1A receptor agonist 8-hydroxy-N-(di-n-propyl)-aminotetralin (8-OH-DPAT). In normal C57Bl6 mice, 8-OH-DPAT induced dose-dependent local changes in relative cerebral blood volume (CBV_{rel}) associated with neuronal activity. In the present work, we analyzed changes in cerebral blood volume (ΔCBV_{rel}) in response to intravenous 8-OH-DPAT administration in adult MSUS and control (CON) mice to investigate whether early life stress alters 5-HT1A receptor function and disturbs the functional connectivity between brain regions known to be involved in the active serotonergic system.

METHODS: Animals were anesthetized with Isofluoran, intubated, placed on a water-heated cradle and artificially ventilated during the fMRI experiment following a previously published protocol [3]. All animal experiments were performed in strict adherence to the Swiss law for Animal Protection.

fMRI: Experiments were performed on a Pharmascan 4.7/16 scanner (Bruker BioSpin GmbH, Karlsruhe, D) operating at 4.7T. The imaging protocol consisted of a spin-echo (RARE) sequence [4] with a spatial resolution: 156x156x700 μm^3; temporal resolution: 40s; TEeff/TR: 80.2/2500ms; field of view (FOV): 2x1.3cm^2; RARE factor: 32; matrix dimension: 128x128; slice thickness: 0.7mm; 8 slices; inter-slice distance: 1.2mm. fMRI experiments comprised 3 phases: 8 images (S_{pre}) were acquired for the determination of CBV changes followed by i.v. administration of the contrast agent (Endorem®, 55mg/kg). After a waiting period of 15 min the fMRI experiment was started: 35 postcontrast images (S(0)) were acquired prior to the i.v. injection of 8-OH-DPAT (0.0-0.1mg/kg for the dose-dependence, N=22) or 0.1 mg/kg for the MSUS (N=10) vs control study (N=10)) followed by a series of 51 images (S(t)). Data analysis: Data analysis was carried out using Biomap (Novartis Institute for Biomedical Research, M.Rausch). Changes of CBV in percent of baseline values ($\Delta CBV_{\%}$) were computed according to $\Delta CBV_{\%}(t)=(\ln\{S(t)/S(0)\})/(\ln\{S(0)/S_{pre}\})*100$. Dose-dependence analysis in C57B6 mice was performed using the mean ΔCBV% of the temporal profile (0-26min). In the MSUS study the temporal profile was analyzed for time x treatment interaction. A cross-correlation-analysis was performed in Matlab using the average temporal profile of 30 ROI calculating correlation coefficient maps for the CON (Fig.4a) and the MSUS group (Fig.4b) and a difference matrix of the correlation maps of the two treatment groups (Fig.4c).

RESULTS: In untreated C57Bl6 mice a dose-dependent decrease in $\Delta CBV_{\%}$, has been observed in a region specific manner reflecting the distribution of the 5-HT1A receptor in the brain. Fig. 1 shows representative activity maps (Fig.1b) and the cortical regions of interest (ROIs, Fig.1a) defined for the quantitative analysis of CBV% changes. For higher 8-OH-DPAT doses the temporal profile of prefrontal cortex (PFC) revealed a decrease of $CBV_{\%}$. Within the placebo group no changes in CBV_{rel} were observed during physiological saline administration (1 ml/kg). Analysis of the integrated $\Delta CBV_{\%}$ (0-26 min after injection) for all doses showed a clear dose-dependence. Application of the highest dose (0.1 mg/kg) to the MSUS treated animals showed a specific decreased response to 8-OH-DPAT. The negative $\Delta CBV_{\%}$ response induced by 0.1 mg/kg 8-OH-DPAT is significantly diminished in the PFC of MSUS mice (Fig.3a), but not in other cortical areas (Fig. 3b,c) or in other brain areas such as the striatum (data not shown) with minimal or no expression of 5-HT1A receptors. Cross-correlation analysis of 30 ROIs covering main cortical and subcortical structures revealed the largest differences in inter-regional correlation coefficients between the CON and the MSUS group in the thalamus (TH), the hypothalamus (HT) and the hippocampus (HC).

CONCLUSIONS: The maternal separation procedure increases helplessness and induces anhedonia, two important depressive-like behaviors. The reduced CBV_{rel} response to 8-OH-DPAT observed in MSUS mice suggests an impaired serotonergic neurotransmission that may result from a reduction in the number or an impaired function of 5-HT1A receptors in the PFC. These alterations may underlie the depression-like phenotype observed in these mice. In fact, clinical neuroimaging studies demonstrated a crucial role of the prefrontal cortex in emotion processing. Changes in various prefrontal cortical regions like the medial and lateral prefrontal cortex have been observed in depression [5]. Application of the described cross-correlation analysis in the MSUS model allows in addition to determine potential disturbances in the connectivity under 5HT1A challenge between brain regions involved in the observed phenotype. The thalamic and hippocampal structures identified in the difference matrix (MSUS-CON, Fig.4c) are among the sub-cortical regions recently identified in a rat fMRI study analyzing correlation maps upon a serotonergic challenge [6].

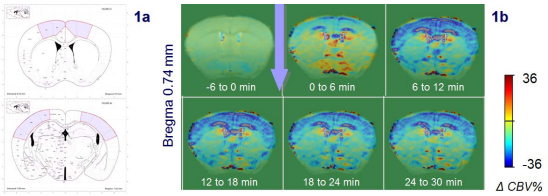

Fig.1: Examples of regions of interest (ROIs) defined for the cortical areas (a) and ΔCBV% maps (superimposed on SE-RARE images) after 0.1 mg/kg 8-OH-DPAT injection at time *t*=0 (arrow) for a control mouse.

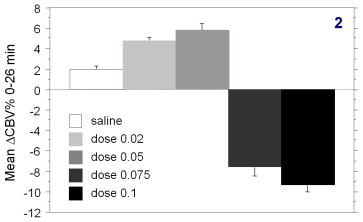

Fig.2: CBV_{rel} in the medial prefrontal cortex plotted as integral over time (0-26 min) indicating a dose-dependent effect of 8-OH-DPAT (0.00, 0.025, 0.05, 0.075, 0.1mg/kg) Statistical analysis revealed a main effect of treatment F (4,16)=12.34 p<0.001. Post-hoc analysis (Fisher's PLSD for ΔCBV%) showed significant differences (p<0.0001) between all doses except for 0.025 vs 0.05 mg/kg.

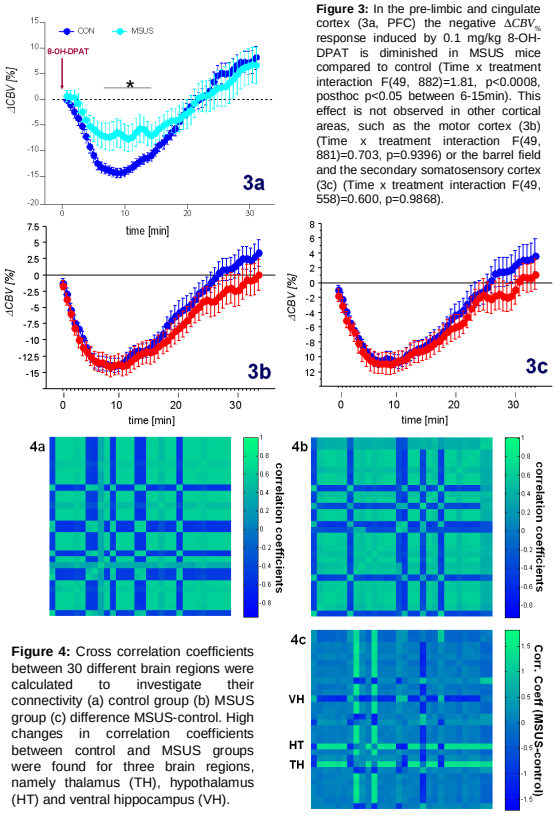

Figure 3: In the pre-limbic and cingulate cortex (3a, PFC) the negative $\Delta CBV_{\%}$ response induced by 0.1 mg/kg 8-OH-DPAT is diminished in MSUS mice compared to control (Time x treatment interaction F(49, 882)=1.81, p<0.0008, posthoc p<0.05 between 6-15min). This effect is not observed in other cortical areas, such as the motor cortex (3b) (Time x treatment interaction F(49, 881)=0.703, p=0.9396) or the barrel field and the secondary somatosensory cortex (3c) (Time x treatment interaction F(49, 558)=0.600, p=0.9868).

Figure 4: Cross correlation coefficients between 30 different brain regions were calculated to investigate their connectivity (a) control group (b) MSUS group (c) difference MSUS-control. High changes in correlation coefficients between control and MSUS groups were found for three brain regions, namely thalamus (TH), hypothalamus (HT) and ventral hippocampus (VH).

REFERENCES:

[1] Heim & Nemeroff et al., Biol Psychiatry, 2001 [2] Stahl, J Affect Disord.,1998 [3] Mueggler et al., Magn Reson Med, 2001 [4] Hennig et al., Magn Reson Med, 1986 [5] Northoff et al., Hum Brain Mapp., 2004 [6] Schwarz et al., Neuroimage 2007

ACKNOWLEDGEMENT: The authors are grateful to the NCCR Neural Plasticity and Repair and Novartis Institute for Biomedical Research, Switzerland for financial support.