Early life stress leads to depressive like symptoms and a reduced response to a 5-HT$_{1A}$ receptor agonist as revealed by pharmacological fMRI in adult mice

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INTRODUCTION Early life stress constitutes a risk factor for the development of psychological disorders, such as depression in human[1]. Early life stress was modeled in C57Bl6 mice using unpredictable maternal separation (daily 3h between post-natal days, PND 1-14) combined with unpredictable maternal stress (MSUS; daily 20 min restraint stress or 5 min forced swimming). MSUS mice tested as adults, exhibited a depression-like phenotype in the Porsolt swim test and anhedonia, a core symptom of depression, in the sucrose consumption test as shown in rats and monkeys[2]. To investigate the role of the 5-HT$_{1A}$ receptor, strongly implicated in the pathophysiology of depression and the action of antidepressant drugs, we established a fMRI protocol for the mouse using the 5-HT$_{1A}$ receptor agonist 8-hydroxy-N-(di-n-propyl)-aminotetralin (8-OH-DPAT).

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

fMRI: Experiments were carried out on a Bruker Pharmascan 47/16 (Bruker BioSpin AG, Karlsruhe, Germany) using the following scan parameters: spatial resolution: 156x156x700 μm^3, temporal resolution: 40s, repetition time: 2500ms, effective echo time: 80.2ms, field of view: 2.0x1.3cm^2, RARE factor: 32, matrix dimension: 128x128, slice thickness. 0.7mm, inter-slice distance: 1.2mm, number of averages: 4, number of slices: 8. The fMRI experiment comprised 3 phases: 8 images (S_{pre}) were acquired as reference for the determination of cerebral blood volume (CBV) changes followed by a bolus administration of the contrast agent (Endorem 55mg/kg). After a waiting period of 15 min the actual fMRI experiment was started: 35 postcontrast images (S(0)) were acquired prior to the injection of 8-OH-DPAT (0.0-0.1mg/kg for the dose-dependence study, 0.1mg/kg for the MSUS vs control study) followed by a series of 51 images (S(t)) documenting the effects of the compound on CBV.

Data analysis: Data analysis was carried out using Biomap (Novartis, M.Rausch). Changes of CBV in percent of baseline values ($\Delta CBV_{\%}$) were computed on a pixel by pixel basis according to: $\Delta CBV_{\%}(t)=(\ln\{S(t)/S(0)\})/(\ln\{S(0)/S_{pre}\})*100$ [Equation 1]

RESULTS In untreated C57Bl6 mice a dose-dependent [0.0, 0.05, 0.075 or 0.1mg/kg 8-OH-DPAT, N=10] decrease in CBV%, calculated according to Eq. [1] has been observed in a region specific manner resembling the distribution of the 5-HT$_{1A}$ receptor in the brain. Figure 2 shows the detailed temporal profile of prefrontal cortex (PFC) ΔCBV% values. In all 3 dose groups a decrease of CBV% was observed 40s after drug administration, peaked at around 4.5 to 6min and slowly increased reaching baseline values (i.e. ΔCBV%=0) 10 min (0.05 mg/kg) and 16 min (0.075 and 0.1mg/kg) after injection of the 5HT$_{1A}$ agonist. Within the placebo group no changes in CBV (i.e. ΔCBV%~0) where observed during physiological saline administration (1ml/kg). Maximum CBV decreases of the order of -25% were measured for the dose of 0.1mg/kg. For the quantitative analysis CBV changes were integrated from 0 to 8min (scan 31-51) for PFC and thalamus (th). Integra ted CBV% changes for the PFC are shown in Figure 3. The corresponding values for thalamus for the doses 0.1, 0.075 and 0.05mg/kg are -170%, -40% and -13%, respectively. Applying the highest dose to the described MSUS model revealed a diminished ΔCBV% reduction in the PFC of MSUS mice when compared to control littermates. Both groups showed region specific changes comparable to the untreated C57/Bl6 mice.

CONCLUSIONS These data show that functional investigation of the 5-HT$_{1A}$ receptor with fMRI is possible in mice using the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT. In addition, the reduced CBV response to 8-OH-DPAT during fMRI suggests that either a reduction of the number of 5-HT$_{1A}$ receptors in the PFC of MSUS mice or an impairment of their function might be involved in the depressive-like phenotype in this mouse model that resembles the etiology and both behavioral and 5-HT$_{1A}$ receptor deficits observed in depressed patients.

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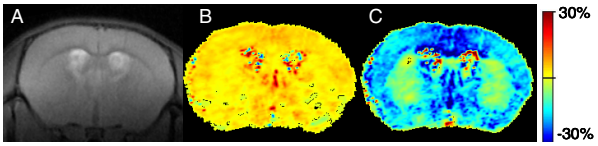

Figure 1: Example of CBV map indicating region specific CBV decreases. **(A)** Transverse structural MR image -0.10 relative to the Bregma. **(B)** Corresponding fMRI baseline map displaying the integrated CBV prior to injection of 8-OH-DPAT (-8 to 0 min). **(C)** Activation map (Integrated CBV change from 0-20 min) showing pronounced CBV changes in the cingulate cortex (Cg1/2) and no changes in the caudate putamen.

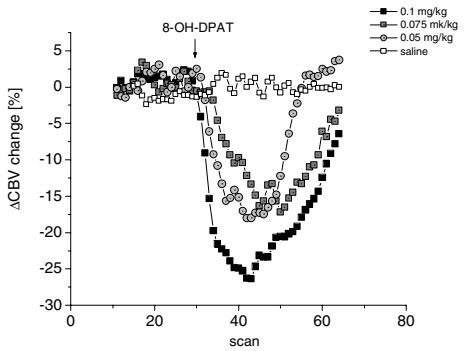

Figure 2: ΔCBV% in PFC depicted from calculated CBV maps as a function of time after injection of 8-OH-DPAT for 3 different doses. At the highest dose used (0.1 mg/kg) maximum CBV% values at the order of -25% can be measured.

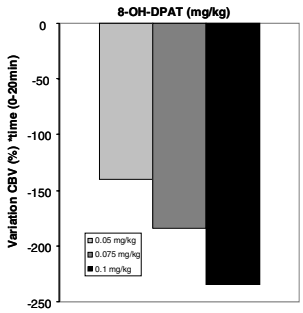

Figure 3: Integrated ΔCBV% response (integration 8 min from time point of injection) in the PFC for 0.05, 0.075 and 0.1 mg/kg.

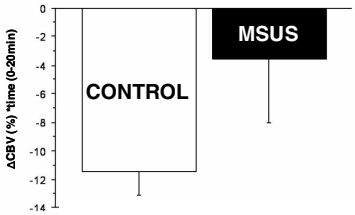

Figure 4: ΔCBV% decrease in the PFC induced by 0.1 mg/kg 8-OH-DPAT is diminished in MSUS treated mice compared to control (Mean±SEM 0-16min following drug administration).