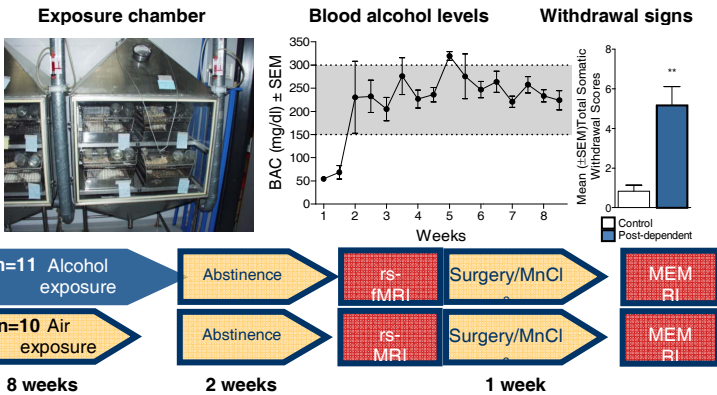


## Altered Brain Connectivity during Abstinence in a rat model of Alcoholism investigated with MEMRI and rsfMRI

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**Purpose:** Discrepancies between disease mechanisms in human and animal models hinder medication development for alcohol use disorders (AUD). In this study we aimed to investigate the brain networks in a rat model of abstinence from alcohol dependence<sup>1,2</sup> with two different approaches for the identification of functional changes. First we acquired resting state fMRI data (rsfMRI) which is a highly translational tool for the measurement of brain connectivity and comparable to human studies<sup>3</sup>. Second we used Manganese-Enhanced MRI (MEMRI)<sup>4</sup> which measures the activation based Mn2+ uptake in localized brain areas. Observed brain signatures are hypothesized to occur in common “AUD-networks” and may be helpful in translating medication effects observed in experimental animals for potential AUD treatments.



**Methods:** 21 Wistar male rats (weight ca. 250g) of which 11 underwent 8 weeks of alcohol exposure to induce dependence (2 weeks of recovery) were scanned at a 9.4T scanner under 0.14 mg/kg/h medetomidine infusion. Additionally to 8.5-min rsfMRI datasets (TR/TE 1700/17.5ms, 29 slices), T1-maps (8 slices with 7TR points) were acquired for each rat (pre). Subcutaneous osmotic pump implantation followed in 6 animals of each group, which delivered continuously 80mg/kg/day Mn2+. One week later, osmotic pumps were removed and a second T1-map scan was conducted in all rats (post). Processing included normalization of all images to a Paxinos standard space template. RsfMRI data was corrected for physiological noise, bandpass filtered and analyzed using ICA (Melodic) with dual regression as well as seed based correlation coefficient (cc) analysis.

Statistical evaluation of T1-difference maps (pre minus post) and rsfMRI cc maps was done with SPM8 second level models.

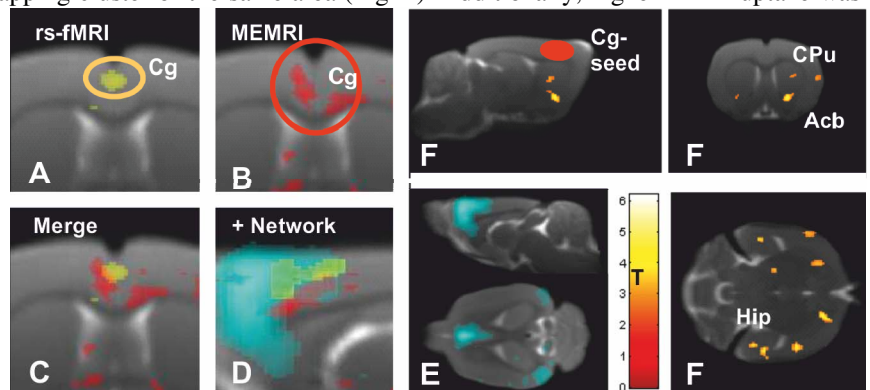
**Results:** Dual regression revealed that the ICA component describing the interoceptive network<sup>4</sup> (anterior cingulate (Cg), insula and visual cortex) was significantly increased in the Cg-area of alcohol dependent rats ( $p < 0.05$ , FWE cluster-corrected). MEMRI also showed significantly higher Mn2+ uptake in an overlapping cluster of the same area (Fig. 2). Additionally, higher Mn2+ uptake was observed in the Putamen (CPu), nucleus accumbens (Acb), Hippocampus (Hip), thalamus and ventral pallidum ( $p < 0.001$  uncor). Using the MEMRI Cg-cluster as seed in a ROI-based cc-analysis also showed additional areas with increased connectivity in the interoceptive network as well as in the putamen (CPu) and nucleus accumbens (Acb).

**Discussion:** After a period of two to three weeks of abstinence we identified altered brain networks by two independent neuroimaging approaches. The identification of the prefrontal cortex corroborates and extends previous research in animal model and alcoholic patients. The implication of the cingulate cortex within the interoceptive network for abstinence and craving remains to be studied.

**Conclusion:** This study provides further evidence that rsfMRI is a sensitive method for the identification of changes in brain networks due to alcohol dependence. Even though both methods rely on fundamentally different approaches to study changes in brain function, we gained strongly overlapping and complementary results.

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**References:** [1] Hermann D et al. *Biol Psychiatry*. 2012; 71(11):1015-21. [2] Meinhardt MW et al. *J Neurosci*. 2013; 33(7):2794-806 [3] Lu H. et al. *PNAS*. 2012; 109(10):3979-8. [4] Lin YJ et al. *Magn Reson Med*. 1997; 38:378-388. [5] Becerra L et al. *PLoS One*. 2011;6(10):e25701.



**Fig.2** Overlapping cluster from rs-fMRI and MEMRI analysis in the anterior cingulate. A,B,C: Enlarged view of clusters identified with ICA (yellow), MEMRI (red) and their overlay on a common template. D: Sagittal view of the same region with the anterior part of the interoceptive network (Cyan) and an overview of this ICA component in E. F: Seed region analysis shows increased functional coupling of anterior cingulate (Cg cluster from B) with striatum, septum, hippocampus and cortical areas including insula, sensory and visual cortex in abstinent rats ( $p < 0.01$  cluster size  $> 60$  Voxel). No regions were found in the opposite contrast.