PhD project description

From MEMRI to MEMORY: a new functional brain imaging technology for the study of learning and memory in behaving mice

Manganese-enhanced magnetic resonance imaging (MEMRI) is increasingly used as a functional MRI (fMRI)-like procedure in freely behaving mice. Because of its unique T1-contrast, MEMRI is sufficiently sensitive to visualize biological processes of e.g., the visual and auditory system\(^1,2\) and brain development\(^3\). Administration of MnCl\(_2\) results in neuronal uptake and retention of paramagnetic Mn\(^{2+}\)\(^4,5\), which acts as Ca\(^{2+}\)-analog and competitively enters active neurons through voltage-gated Ca\(^{2+}\) channels. Such Ca\(^{2+}\) channels are pivotal for cellular mechanisms underlying learning and memory processes. Upon uptake, Mn\(^{2+}\) will remain sequestered for days within previously activated neurons, and as such can be used as a tool to uncover brain activation patterns underlying learning and memory processes.

The objective is to visualize and map neuronal activation patterns underlying key components of learning and memory phases using MEMRI in behaving adult mice.

For this purpose, a fear conditioning task requiring differential activation of brain areas such as amygdala, hippocampus, and prefrontal cortex will be implemented. Groups of mice will receive a systemic MnCl\(_2\) injection and undergo MEMRI. Patterns of brain activity that are specific to key components of learning and memory phases (e.g., acquisition, retention) will be deducted by comparing MEMRI measurements obtained at critical time points following onset of behavioural testing.

We expect that learning processes will change the basic pattern of T1-enhancement in the brain. The results of this pilot study will greatly contribute to pioneer MEMRI as the new generation of fMRI methodology applicable to the study of cognitive processes in mice.

References:


This project is part of the research program of Prof. Dr. M.S.Oitzl on “Stress, emotional memories and their extinction”. From MEMRI to MEMORY will be a collaborative PhD-project of Prof. Dr. M.S. Oitzl (LACDR, Division of Medical Pharmacology) and Dr. L. van der Weerd (LUMC, Radiology). Dr van der Weerd is the coordinator of the Leiden Animal MRI facility. Her expertise ranges from MRI and MRS application in healthy small animals (rats, mice) to models of (neurological) diseases.
Experiments and Methods

This project is a multidisciplinary approach combining research including behaviour and brain imaging.

**Behaviour:** Mice will be tested in a fear conditioning paradigm. A series of electric foot-shocks will be associated with a tone. Returning the mice into the same environment or presentation of the tone alone will result in behavioural immobility, which presents fear learning.

The dose of MnCl$_2$ will be partially based on the literature studies and injected 24 hrs before subjecting mice to the test box. Mice will either receive a shock or be allowed to explore the test box without shock (sham condition).

**Imaging and Analysis:** Mice will undergo MEMRI at several time points that were chosen to delineate key components of learning and memory: from acquisition to retrieval. These are investigated according to a specified schedule.

*Representative MEMRI: Pattern of neural activity is depicted from a mouse brain (horizontal plane) under basal condition, 16 hrs after injection of 4 mmol/kg MnCl$_2$ i.p. Note the enhancement of activity in the dentate gyrus (DG) and CA3 hippocampal subregion and olfactory bulb (OB) (courtesy Dr. L. van der Weerd).*

Note that MEMRI reflects MR contrast enhancement in brain regions with higher uptake of Mn$^{2+}$. We will start with the transversal approach which compares animals that are behaviourally tested and subjected to MEMRI at specified time points to differentiate cognition-specific from other activity.

**Equipment for Imaging and Analysis:** Leiden University has two ultra-high field MRI systems (Bruker 9.4T and 17.6T magnets equipped with Bruker Micro gradient systems of 1 T/m) allowing for in vivo imaging with high spatial (40 µm) or temporal (1-30 s) resolution. A 7T horizontal-bore animal MRI system (Bruker Pharmascan) is operative at the LUMC.

MEMRI data from each mouse brain will be extracted, co-registered and averaged per group using the software packages developed by Dr. Behloul. Within each averaged 3D dataset, enhancement patterns will be quantified, histograms will be analyzed and enhanced regions will be colorized and displayed in surface renderings representing the volumetric regions of activity.

**References:**
