The dose makes the poison - Studying toxicity in MEMRI applications

B. Gruenecker[1], S. F. Kaltwasser[1], Y. H. Peterse[1], P. G. Saemann[1], M. Schmidt[1], C. T. Wotjak[1], and M. Czisch[1]
[1]Max Planck Institute for Psychiatry, Munich, Germany

Introduction

Manganese-enhanced MRI (MEMRI) is an increasingly used imaging method in animal research, which allows improved T1-weighted tissue contrast, thus enabling *in vivo* visualization of neuronal activity[1, 2]. At higher concentrations, however, manganese (Mn^{2+}) exhibits toxic effects that interfere with the animals' behaviour and well-being. While CD1 mice tolerate single doses of ~100mg/kg $MnCl_2$ well, C57BL/6N show severe distress. Therefore, a trade-off has to be made between minimizing side effects and gaining high image contrast when optimizing MEMRI protocols. Recently, a low concentrated fractionated Mn^{2+} application scheme has been proposed for rats as a promising alternative[3]. Here, we investigate the potential of such a fractionated application protocol for the widely used mouse strain C57BL/6N.

Methods

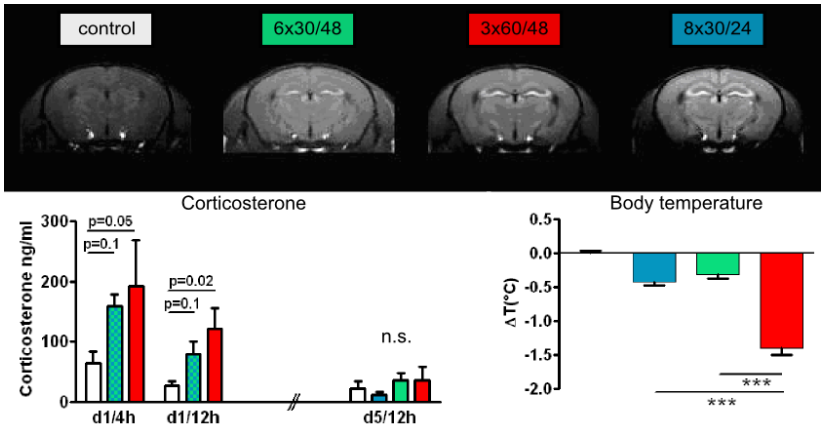
We investigated effects of different fractionated Mn^{2+} dosing schemes on vegetative, behavioural and endocrine markers of animal's well-being as well as stress levels, and MEMRI signal contrast in C57BL/6N mice. Measurements of animals' well-being included telemetric monitoring of body temperature and locomotion, weight control and observation of behavioural parameters during the time course of the injection protocols. As endocrine marker of the stress response we determined corticosterone levels after $MnCl_2$ application.
We compared three $MnCl_2$ application protocols: 3 times 60 mg/kg every 48 hours, 6 times 30 mg/kg every 48 hours, and 8 times 30 mg/kg every 24 hours (referred to as 3×60/48, 6×30/48 and 8×30/24 respectively).

Results

Both, the 6×30/48 and the 8×30/24 protocols showed less negative effects on animals' well-being. Highest MEMRI signal contrast was observed for the 8×30/24 protocol compared with the 3×60/48 and 6×30/48 protocol.

Discussion

Results argue for a fractionated application scheme such as 8 times 30 mg/kg every 24 hours to provide sufficient MEMRI signal contrast while minimizing toxic side effects and distress. More specifically, we demonstrate that behavioural, vegetative and endocrine markers of stress are only minimally and transiently affected, when a fractionated application scheme with low single doses is used. Furthermore, we provide evidence that higher total doses of Mn^{2+} lead to improved general and between-region MEMRI contrasts (see figure).
Our results indicate that thorough observation beyond the level of mere visual inspection is required to reliably map animal's distress. MEMRI with fractionated Mn^{2+} applications opens the possibility to study complex behavioural paradigms that probe the animals' stress system in freely behaving animals, as only the read-out of the cerebral Mn^{2+} accumulation requires sedation. When studying sensitive mouse models with potentially compromised health (e.g. genetically modified animals or mice at early developmental stages) the fractionated approach may be particularly useful to avoid unnecessary stress for the animal.



Top: Coronal slices for control, 6×30/48, 3×60/48 and 8×30/24 application schemes. Mean images of the different groups are shown. Higher total dose of Mn^{2+} improves general and between-region contrast.
Bottom: HPA-axis response to different doses of delivered $MnCl_2$ (left). Body temperature deviation from a three day baseline (right).

Reference List: (1) Weng J.C. et al. (2007) NeuroImage **36**, 1179-1188. (2) Lu H. et al. (2007) *Proc. Natl. Acad. Sci. U. S. A* 104, 2489-2494. (3) Bock N.A. et al. (2008) NMR in Biomed. 21, 473-478.