Continuous infusion of manganese improves contrast and reduces side effects in MEMRI studies

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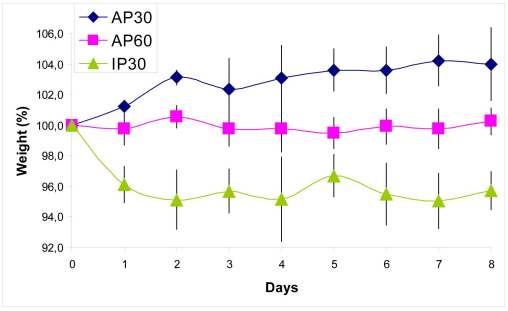
Introduction The accumulation of manganese in firing neurons enables the visualization of activated brain regions and has been widely used to monitor brain activity by means of manganese-enhanced MRI (MEMRI). However, few studies until now have used MEMRI for general phenotyping of transgenic animals. The main challenge in this case is administering a high enough dose of manganese while avoiding systemic toxicity, which is particularly problematic in (transgenic) mice. Recent studies have been successful in achieving enhancement by daily administration of a low dose (30mg/kg) either via intra-peritoneal (i.p.) injections [1] or by infusion via osmotic pumps (o.p.) [2]. To ensure the acquisition of 3D images of high quality and contrast, while allowing a short acquisition time via reduced tissue T1 values which are critical for phenotyping a relatively large number of transgenic animals, it is necessary to employ the highest dose without increasing the side effects. In the present study, we investigate the possibility of administering a daily dose of 60 mg/kg while circumventing its side effects.



commencement of daily administration of 30mg/kg ip (green triangles), 30mg/kg infusion (blue diamonds) and respectively 60mg/kg infusion (pink squares). The weight is expressed as percentage of the weight before treatment. The error bars represent the standard error.

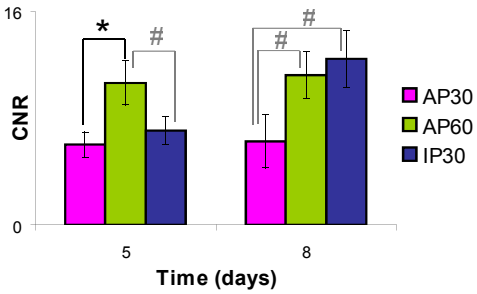


*Figure 2. Average CNR for IP30 (green), AP30 (blue) and AP60 (pink) between hippocampus and cortex after 5 and respectively 8 days of manganese treatment. *: $p<0.05$; #: $p<0.1$.*

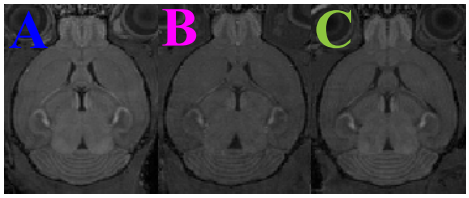


Figure 3. Example of contrast acquired after a cumulative dose of i.p. injected 240mg/kg (A), infused 240mg/kg (B) and infused 480mg/kg (C).

Materials and methods *Contrast agent:* Groups of 4 C57Bl6J mice were administered daily doses of $MnCl_2$ for 8 days, as follows: Group IP30 received 30mg/kg $MnCl_2$ intraperitoneally, and groups AP30 and AP60 received 30mg/kg and 60mg/kg $MnCl_2$ respectively, via Alzet infusion pumps. This resulted in cumulative doses of 240mg/kg $MnCl_2$ in groups IP30 and AP30, and 480mg/kg $MnCl_2$ in group AP60. The mice were monitored daily for weight loss and signs of discomfort. *Imaging:* Whole brain 3D images were acquired using a T1-weighted gradient echo sequence in a 7T horizontal bore Bruker Pharmascan. The MR acquisition protocol consisted of 45 min scans (TR=50 ms, TE=3.2 ms, spatial resolution =0.140x0.125x0.125 mm^3, matrix size=128x104x104 zero filled to 128x128x128, FOV=17.92x16x16 mm, 5 averages). Scans were acquired for each mouse at three different timepoints: before the start of manganese treatment, and 5 days and 8 days after the beginning of the treatment. A tube filled with 1mM $CuSo_4$ in water was used as reference specimen. *Processing:* The three scans acquired per animal were rigid-body registered to each other using the ITK-based publicly available Elastix software [3], to allow an automatic voxel-by-voxel calculation of the contrast enhancement. The contrast to noise ratio (CNR) was calculated as follows: $CNR = (SI_{hippocampus} - SI_{cortex})/StDeviation_{Noise}$.

Results and discussion The i.p. injected $MnCl_2$ treatment lead to a significant ($p<0.0001$) weight loss of 4.3±2.1 % within two days, followed by a stabilization of the weight. These mice also displayed matt fur, indicating a certain degree of discomfort. The two infusion groups showed no signs of discomfort and no significant weight loss was registered (Fig. 1), suggesting that a slowee administration avoiding plasma peaks is beneficial. The achieved SNR with our T1-weighted protocol was comparable between all groups (data not shown). Group AP60 showed a significantly higher CNR after 5 days of treatmentand a CNR comparable to group IP30 after 8 days (Fig. 2 and 3). It is likely that the increased exposure to $MnCl_2$ which is inherent to i.p. administration leads to higher accumulation of Mn^{2+} in the entire brain, thereby reducing the contrast.

Conclusion Administering $MnCl_2$ via infusion pumps allowed delivering the highest dose attempted so far – 480mg/kg $MnCl_2$ – to C57Bl6J mice. This dose achieved a high contrast and circumvented the weight loss or visible signs of distress seen in the mice which received manganese via fractionated i.p. injections. This manganese administration protocol will be of particular use to study brain activation patterns occurring over several days, such as in transgenic mouse models, or chronic disease models.

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[2] Mok S, et al, *Brain Struct Funct*, 2011
[3] Klein S, *IEEE TMI,* 2010