An improved activity-induced manganese-dependent MRI study of the rat barrel cortex

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Purpose: In 1997, Lin et al. (1) proposed an alternative to BOLD functional MRI to image brain function using Manganese. The paramagnetic properties of Mn^{2+} ions allowed for the first time the indirect measurement of neuronal activation through activity-induced manganese-dependent (AIM) MRI. However, the technique highly depends on the permeability of Mn^{2+} ions through the blood brain barrier and the minimization of their toxicity. Aoki et al. (2,3) further described the potential of AIM MRI and ways to overcome these difficulties. Nonetheless, although the technique is very attractive and promising for direct monitoring of regional brain activation independent of hemodynamic changes, it remains difficult and has not yet been extensively used. Here, we propose an optimized AIM MRI investigation of the rat somatosensory cortex under tight physiological follow up and an histogram analysis for an increased characterization of the barrel cortex synaptic activity.

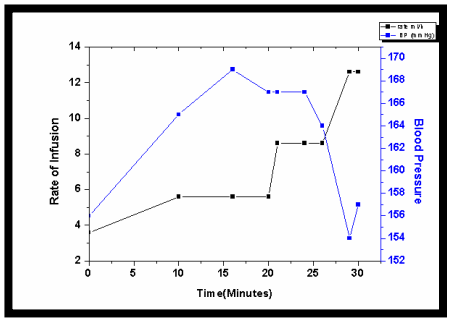
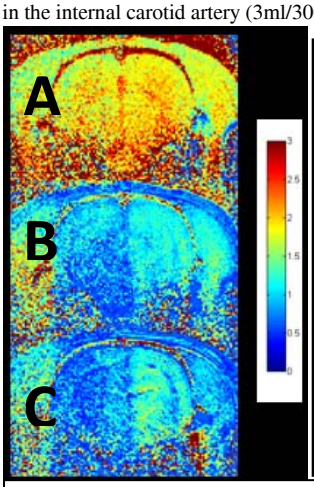


Figure1: Evolution of mean blood pressure (mmHg) and rate of infusion (ml/h) during $MnCl_2$ infusion

Materials and Methods : Animal preparation: 6 Male adult Sprague-Dawley rats (350±40g) were orally intubated. The femoral arteries and veins were catheterized for α-chloralose administration (Bolus 80mg/kg and continuous i.v infusion at 27mg/kg/h), blood gas sampling and $MnCl_2$ infusion. **Carotid surgery:** Blunt dissection alongside the left carotid artery to expose the carotid artery bifurcation into the internal and external branches was performed followed by a ligature of the external carotid artery. The common carotid artery was then exposed. A home-made T-shaped catheter was prepared using PE50 polyethylene tubing. One end was inserted in the internal carotid artery and the other end was inserted in the common artery. Both ends were secured with suture. Blood re-circulated in the catheter loop restoring carotid artery circulation. The long end of the loop catheter was filled with heparinized saline until mannitol injection. After fixing the rat head using ear and bite bars, the rat was positioned in a dedicated holder. The breathing rate was monitored simultaneously with body temperature throughout the experiment with a rectal probe (37.5ºC ± 0.5ºC).The blood pressure was monitored through a transducer attached to the catheterized femoral artery.

$MnCl_2$ and Mannitol Administration: A 100mM standard solution was diluted to 25mM using 0.9% saline. The solution was constantly infused into the rat femoral vein with a syringe pump at a rate starting at 3.6ml/hour. If the blood pressure stayed stable during a 500µl infusion of $MnCl_2$ then the rate of infusion was increased by 2ml/hr up to a maximum rate of 18ml/hr until 8.5-10ml of $MnCl_2$ were infused. A solution of 25% D-mannitol was manually injected through the loop catheter inserted in the internal carotid artery (3ml/300g). Mannitol injection was performed with the rat holder outside of the magnet and while again following carefully the breath rate



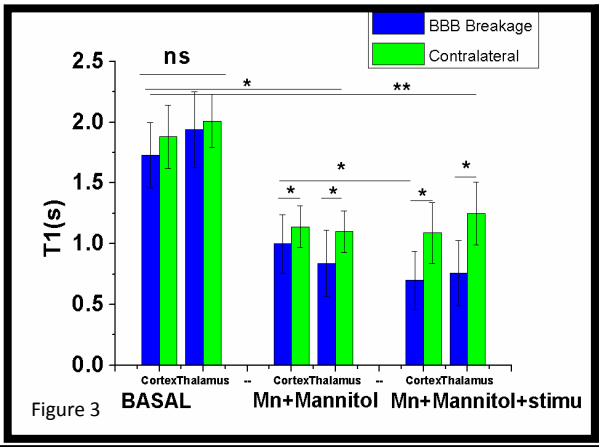


Figure 3

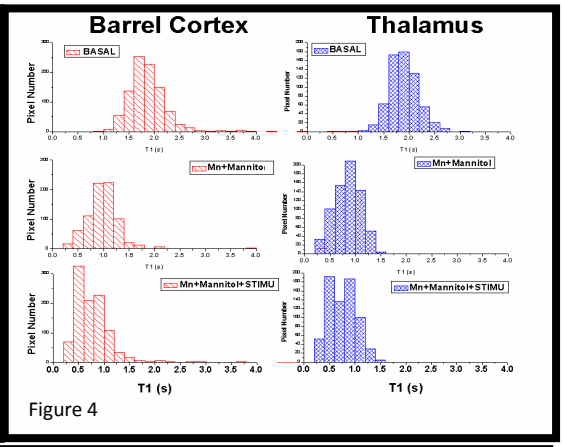


Figure 4

Figure2: T1 SRTF maps : A. Basal. B After Mn+ Mannitol infusion. C. After Mn+Mannitol + 10 min TGN stimulation. **Figure 3**: Comparison of mean T1 values in ROIs placed in the somatosensory cortex and the thalamus of the BBB broken side and the contralateral side. **Figure4**: T1 distributions within each ROI of the BBB broken side in the bsal condition , after Mn+ mannitol infusions and after TGN stimulation

and the blood pressure known to increase after mannitol injection. As the blood brain barrier remains permeable to manganese for a period ranging from 5 to 35 minutes, care was taken to proceed rapidly for further scanning procedures. **Manganese-Enhanced MRI:** For T1-weighted images at 9.4T (Varian), gradient echo 3D images (or MPRAGE) were acquired with the following parameters: TR/TE=10/23ms, Flip angle=90º, FOV=21x21x21mm^3, matrix size=128x128x32 or 64x64x32, coronal slices, BW=24 KHz, 3-4 averages. For T1 quantification, a 2D saturation-recovery TurboFLASH sequence was used (4) (TR/TE=200/3.1ms, flip angle=45º, Recovery time (TSR), TSR=0.02-14s, step=0.5s, 2-6 slices, FOV=21x21mm^2, Matrix size=64x64 or 128x128, TH=1mm). Images were acquired before $MnCl_2$ infusion, after 5ml infusion of $MnCl_2$, after Mannitol injection and after 10-15 minutes trigeminal nerve stimulation (5).

Results and Discussion: Fig1 shows the evolution of blood pressure (mmHg) during the infusion of MnCl2 at an evolving rate (ml/h) for a single rat. The physiology of the rat was carefully followed during the infusion of all compounds (PCO2=38-45mmHg, T=37.5-38º, MABP=150-180mmHg, pH=7.3±0.1, Heart Rate=369.6±24 bpm). During MnCl2 infusion, each time blood pressure dropped by more than 10%, the infusion was stopped and restarted when all the parameters were stabilized. After injection of Mannitol, the BBB was broken and MnCl2 slowly diffused into the thalamus and cortical areas as shown in T1 pixel-by pixel maps (Fig2.A and 2B). Upon 10min TGN stimulation, MnCl2 further diffused into the barrel cortex were T1 further dropped (Fig2C). T1 values were evaluated over ROIs in the thalamus and the somatosensory cortex (Fig3) before $MnCl_2$ infusion, after $MnCl_2$ and Mannitol infusion and after TGN stimulation showing significant changes between the BBB broken side and the contralateral side of the rat brain and between each condition (paired t-test, *p<0.05, ** p<0.01). The T1 distributions over each ROI were also compared (Fig4) showing a significant shift of T1 values in the range 1-3s in the basal condition to 0.2-1.5s after $MnCl_2$+ Mannitol and after stimulation whether in the somatosensory cortex or the thalamus. Within the barrel cortex, the number of pixels with T1s in the range 0.2-0.6s was multiplied by 5 after the stimulation step.

Conclusion: With a precise follow up of physiological parameters during $MnCl_2$ and mannitol infusions, the reproducibility of AIM MRI protocol is increased. Our methodology allowed T1 mapping and indirect mapping of calcium-dependent synaptic activation within the barrel cortex. With histogram analysis we hope to further characterize the synaptic activity with an improved localization of cortical layers.

References: 1. Lin et al. MRM 38:378-388,1997. 2. Aoki et al.NMR in Biomed. 17, 569-580, 2004.3.Duong et al.MRM 43:383-392,2000. 4. Parker GJM et al. MRI 18:157-167, 2000.5. Just et al. MRI 28 :1143-1151, 2010.