Logan graphical analysis for quantitative evaluation of Calcium channel activity in the pituitary gland using manganese-enhanced MRI (MEMRI)

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Introduction: The purpose of this study was to quantitatively evaluate Ca^{2+}-channel activity in the pituitary gland (Pit) using manganese-enhanced MRI (MEMRI) and Logan graphical analysis (LGA). LGA is an algorithm already widely used with positron emission tomography (PET) for the quantitative analysis of *reversible* processes such as ligands binding temporarily to neuroreceptors [1]. When adapted to MRI studies where a contrast agent is used as a tracer that enters tissue *reversibly*, LGA states that there is a certain time t^* after tracer administration where a plot of $\int C_t/C_t$ versus $\int C_p/C_t$ becomes linear (C_t and C_p are the concentrations of the tracer in tissue and arterial plasma, respectively). As an example, for a two-compartment model, the slope of the linear relationship provides direct information about the inflow and outflow constants of the particular tracer from plasma to tissue.
Since manganese ions (Mn^{2+}) can enter excitable cells through Ca^{2+} channels, Mn^{2+} ions have been used as a MRI contrast agent for both functional and anatomical studies [2]. However, because the blood brain barrier (BBB) hinders the passage of Mn^{2+} ions into brain tissue when using manganese-enhanced techniques such as AIM-MEMRI [3], the BBB must be artificially disrupted so that neural activity can be observed. On the other hand, it is well known that Mn^{2+} ions can enter the central nervous system in circumventricular organs such as the Pit because the BBB is nonexistent or leaky.
In this study, the Mn^{2+} concentrations in the posterior pituitary gland (PPit) were measured with rapidly acquired T_1-maps before and after contrast agent injection [4, 5]. The Ca^{2+}-channel activity was varied by administration of excitatory (Glutamate) and inhibitory (Verapamil) agents and the difference in channel activity was evaluated using LGA.

Method: All experiments were performed on a 7T animal MRI system (Magnet: Jastec, Japan, Console: Bruker Biospin, Germany). Manganese infusion was performed on 3 groups of male Wistar rats. The Baseline group received only a 0.5ml/100g body-weight bolus-injection of a 10mM osmotic pressure-controlled $MnCl_2$-saline solution. The Glutamate group received a 0.5ml/300g body-weight bolus-injection of a 5 mg/ml Glutamate-saline solution together with the $MnCl_2$-injection. The Verapamil group received 3 µl of a 5mg/2ml Verapamil solution administered via the left nasal cavity 30 min prior to the $MnCl_2$-injection. A scout scan was performed to locate the pituitary gland, after which several T_1-maps were acquired with a Look-Locker-EPI sequence (TE=4.2 ms, TR=10000 ms, 64 shot) prior to $MnCl_2$-injection. After $MnCl_2$-injection, a series of T_1-maps were acquired over a period of 130 min. Over the same period, sixteen 100 µl blood samples were taken. The T1s of the blood samples were measured after the in vivo experiment was completed.
Mn^{2+} concentration in the PPit (Figure 1) and plasma were estimated with the relation $C = (1/T_{1,ca} - 1/T_{1,0})/r$, where $T_{1,ca}$ is the T_1 after contrast-agent administration and $T_{1,0}$ is the T_1 prior to contrast-agent administration. The relaxivity of the contrast agent, r, and was taken to be 6.5 $s^{-1}mM^{-1}$ in tissue and 0.8 $s^{-1}mM^{-1}$ in blood.

Results: Figure 2 shows the average Mn^{2+}-concentration of each group as a function of time in tissue and plasma. The concentration varied depending on stimulant/inhibitor administration. The difference in peak concentration for tissue and plasma just after administration suggests that the influx constant of Mn^{2+} into the tissue is highest for the Glutamate group and lowest for the Verapamil group. Figure 3 shows a LGA applied to the averaged data of each group. For a t^* around 40 min the plot clearly becomes linear for all datasets. The slopes of the Glutamate and Verapamil data are larger and smaller, respectively, when compared to the slope of the Baseline data. This difference in slope is consistent with the previously suggested difference in Mn^{2+} influx constants.

Discussion: As can be seen from the data, it is possible to obtain information about the inflow and outflow of Mn^{2+} ions in the PPit with LGA and MRI. LGA was also performed for a region covering the Anterior Pituitary Gland (Fig 2, APit, data not shown). However, no t^* was found for that region, indicating that LGA cannot be applied for the APit. In that case, an analysis suitable for the *irreversible* influx of contrast agent into the tissue should be applied.
For tissues such as the PPit, however, where the outflow of contrast agent is not negligible, application of the LGA is feasible and can deliver valuable insight into the effects of excitatory and inhibitory agents on Calcium channel activity.

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References: [1] Logan J. et al: JCBFM, 1990; 10:740-747 [2] Aoki I. et al: MRM, 2002: 48:927-933 [3] Lin YJ. and Koretsky AP: MRM, 1997; 38:378-88 [4] Chuang K. et al: MRM, 2006; 55:604-611 [5] Skjold A. et al: JMRM, 2006; 24:1047-1055

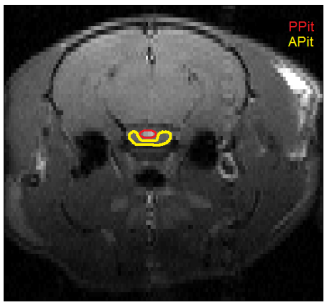

Figure 1: Axial slice showing the Pituitary Gland with the (red) PPit and (yellow) APit.

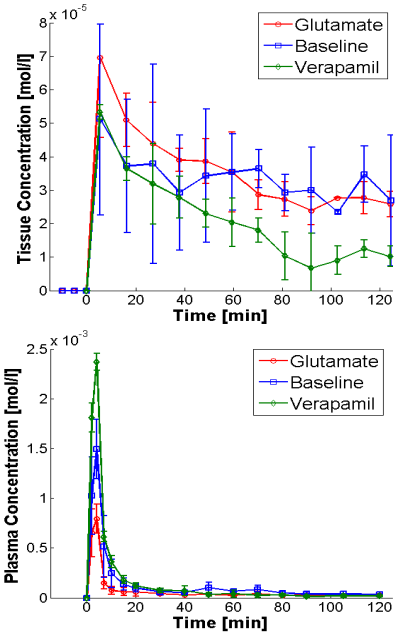

Figure 2: (top) Averaged Mn-concentration measured in tissue versus time. (bottom) Averaged Mn-concentration measured in blood plasma versus time.

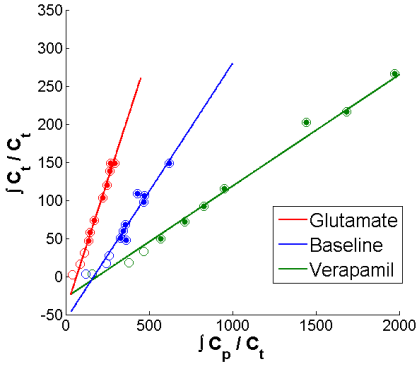

Figure 3: LGA applied to the averaged data of each group. The filled circles are the data points acquired after t^* and were used for linear fitting.