

Intracerebroventricular Infusion of Manganese via Osmotic Pump

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Introduction: Manganese-enhanced Magnetic Resonance Imaging (MEMRI) is a promising technique for functional brain imaging in live animals (1). Studies have addressed that activation in awake moving animals can be monitored by performing MRI after the presumed activity has occurred, when preceded by an intraperitoneal injection of manganese (Mn) (2). Since the discovery of MEMRI, researchers have used many techniques to administer Mn including; intraperitoneal injections (3,4), tail-vein injections (5) and subcutaneous osmotic pumps (4). Direct intra-cerebral injections of Mn have been performed using small bolus injections (6,7) or via osmotic pump connected to cannulae (7). In this study we investigated the use of intra-cerebro-ventricular cannulation with the use of an osmotic pump to administer a continuous dose of $MnCl_2$ and its effect on the tissue and behaviour.

Methods and Methods: Four Long-Evans female rats (120 days old and weighing 200-400g) were studied. Bicine buffered Mn was delivered via a cannula connected to an osmotic pump (ALZET., California). Two Mn infusion rates were tested 1) (7 day pump: 0.85mM 1ul/hr 200ul) or 2) (28 day pump: 100mM 0.25ul/hr 200ul). Control rats were infused with a solution of bicine (pH 7.4) for each of the 7 or 28 day pumps. *Cannulation for the Injection of $MnCl_2$:* Left or right lateral ventricle (depending on handedness) was the target for the intracranial cannulation. Coordinates were + or -2.7mm lateral, -1.72mm bregma and 3.7mm ventral to zero line. Rats were given a 3 day recovery period prior to behavioural tests and imaging. *Single Pellet Reaching Behavioural Task:* The analysis of the Single Pellet Reaching Task included quantitative and qualitative measures identified using the Eshkol-Wachman Movement Notation (8). The rats were pre-trained in the reaching task, received surgery to insert the cannula and pump (containing $MnCl_2$ or Bicine) and then they were tested every weekday for up to 4 weeks depending on pump duration. *Activity Box:* Rats were placed in the activity boxes (Accuscan Instruments Inc., Ohio) once a week to measure general activity level by assessing motor and exploratory activities. All subjects had access to food and water *ad libitum*, except during the single pellet reaching task, in which animals were placed on food deprivation while maintaining 85% of their body weight. *Imaging:* Following recovery, imaging was performed every second day using a 4.7 T 330 mm bore Oxford magnet and a SMIS console. A two RF coil system consisting of a quadrature birdcage transmit coil (10cm in diameter, 10 cm in length, Morris Instruments), a homebuilt receive only butterfly coil (2cm in diameter) and PIN diode switching was used. Locator images (SE-TR/TE 700/13 ms 0.2x0.2x1.5mm) were followed by a 7 image saturation recovery SE (TE=22, TR = 100-4000ms 0.39x0.39x1.5mm) T1 mapping data set. Signal intensity from ROI's were extracted from the T1 images with Analyze 8.1 (BIR, USA) and T1 values were calculated using home written software written in IDL.

Results: *Imaging:* After 10 days post-surgery, a black region began to appear in the brain of the 28-day $MnCl_2$ Rat. Using cresyl violet histology we confirmed that this was a lesion. Figure 1 shows the corresponding stained brain slices to the MRI images. *T1 Maps:* Figure 2 shows the T1 maps demonstrating a clear change in the T1 values. *Single Pellet reaching task:* The results for the single pellet-reaching task demonstrated that the $MnCl_2$ had no effect (P=0.68 for 28-day and P=0.5 for 7-day) on the fine motor skills demonstrated by the rat. *Activity Box:* Measurement of spontaneous motor activity showed that the $MnCl_2$ had no effect on locomotor activity.

Discussion: Our results show that in all cases, (7 and 28 day) the behaviour was not significantly affected by the infusion. From the histology, it is clear that the 28-day pump using a concentration of 100mM with an infusion rate of 0.25uL/hr will cause cell damage. The MRI images indicate that this damage occurs after about 10 days. The low dose experiment indicates that Mn can be delivered

without cell damage at a concentration of 0.85 mM at rate of 1ul/hr over 7 days. The control experiments do not show significant cell damage confirming that the cause was due to the Mn in the solution. The low dose delivery produces a clear relaxation time reduction.

References:

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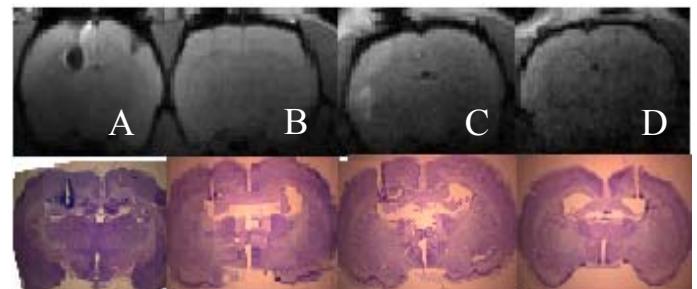


Figure 1. Coronal images of (A) 28-day $MnCl_2$ (B) 28-day bicine control (C) 7-day $MnCl_2$ (D) 7-day bicine control. The histology corresponding to each image is presented underneath.

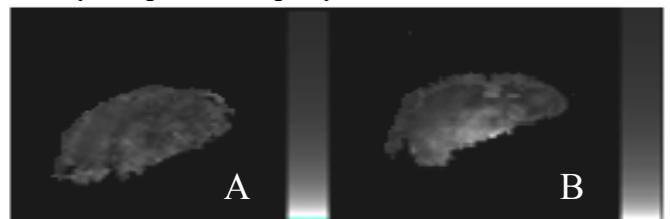


Figure 2. Coronal T1 maps of (A) 7-day bicine Rat (B) 7-day $MnCl_2$ Rat (intensity bar is for relaxation rate for T1 between 200ms and 2000ms). The lower right part of the brain is truncated due to the coil sensitivity and the noise threshold criteria of the T1 mapping.