In vivo manganese-enhanced MRI (MEMRI) of the appetite regulations, correlation with c-fos expression

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Introduction: Obesity is currently a major health problem across the world. The mechanisms underlying the development and maintenance of obesity are not fully understood, although patterns of eating are undoubtedly altered in obesity. Manganese-enhanced MRI (MEMRI) has been proposed as a potential method to delineate neuronal connectivity and to assess brain structures in experimental animals[1, 2]. An in vitro technique, c-fos expression, has helped to identify some of the regions of the brain activated during hunger and satiety. However, the effect of manganese on the brain activation has not been determined with c-fos expression. In this study we have used MEMRI to determine the neuronal activations associated with appetite in a murine model, using ghrelin and PYY_{3-36}. We also performed c-fos expression induced by ghrelin with co-administration of manganese.

Methods: Male C57BL/6 mice (16 – 24 weeks old) were anaesthetized with isoflurane-oxygen mixture and scanned on a 9.4T horizontal-bore MR scanner (Varian, USA) before and after tail vein intravenous (IV) administration of 62.3 mM $MnCl_2$ ($MnCl_2{\cdot}4H_2O$, 5 µl/g) and intraperitoneal (IP) administration of ghrelin (0.06 and 0.30 nmol/g; N = 5 and 4, respectively), or the vehicle solution (PBS; N = 4) to fed mice and PYY_{3-36} (100 µg/kg; N =5) or PBS (N =5) to fasted mice. Spin-echo multi-slice T1-weighted sequence was carried out with following scanning parameters; repetition time (TR) = 600 msec, echo time (TE) = 18 msec, matrix = 256 x 192, field of view (FOV) = 25 mm x 25 mm, 1 average, 10 transverse slices and 1 mm thickness. The scanning time for each acquisition at each time point is 1 min 57 sec. After 3 baseline scans, ghrelin, PYY_{3-36} or PBS was injected through an IP catheter with simultaneous IV administration of $MnCl_2$ administration at a rate of 0.2 ml/hr, and 63 scans continuously recorded for 2 h. The signal intensities (SI) of arcuate hypothalamic nucleus (Arc), periventricular hypothalamic nucleus (Pe), ventromedial hypothtalamic nucleus (VMH), paraventricular hypothalamic nucleus (PVN), anterior pituitary gland (AP), 4th ventricle and phantom filled with saline were measured by drawing region of interests (ROI) with a imaging processing software (Image J 1.3.1, NIH, US). The normalized SI (SI of target area/SI of water phantom) at each time point was obtained, and a time-course of SI change in various areas created (Prism 4, GraphPad Software, CA). The c-fos expression was performed in mice administered with ghrelin with manganese (N = 4), ghrelin without manganese (N = 3), PBS with manganese (N = 4) and PBS without manganese (N = 4). ANOVA was used to compare the difference between different groups, and a $P < 0.05$ was considered statistically significant.

Results & Discussions: The time-course of enhancement for the different brain regions differed following IP neuropeptides or PBS injection. Fasting also increased significantly MEMRI enhancement in hypothalamic nuclei. Administration of ghrelin led to significant modulation in the time-curve of the $MnCl_2$ enhancement, which appeared to be region specific. In the Arc (Fig 1) and Pe, significantly higher enhancement was observed after $MnCl_2$ infusion in the presence of ghrelin at both doses compared to PBS ($P < 0.001$). However, no significant difference ($P > 0.05$) was observed between different doses of ghrelin. In the VMH (Fig 2), a dose response modulation of the Mn^{2+} associated increase in SI ($P < 0.001$) was observed. In PVN, mice injected with high-dose ghrelin showed higher SI than low-dose and control groups ($P < 0.001$). In addition, PYY_{3-36} led to decrease enhancement in Arc as compared to fasted controls, while no significant modulation of manganese enhancement in VMH. PYY3-36 also significantly decreased the enhancements in Pe and PVN ($P = 0.001$) at the steady-state. No significant difference in SI was observed in AP and in the 4th ventricle ($P > 0.05$). The c-fos expression showed significant increase of the activated neurons in Arc in the presence of ghrelin. Administration of the manganese did not change c-fos expression significantly (Fig 3 and 4).

Fig 1

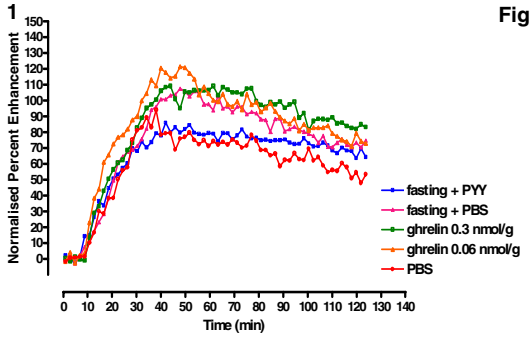


Fig 2

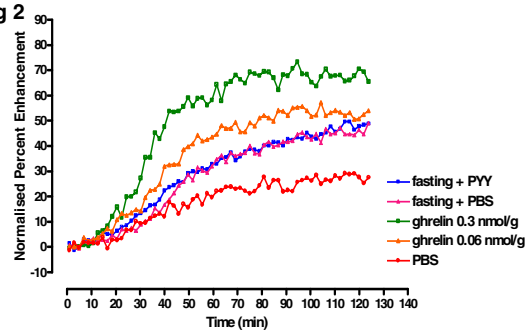


Figure 1. The time-course enhancement in Arc following peptides and PBS administration.

Figure 2. The time-course enhancement in VMH following peptides and PBS administration.

Figure 3. c-fos expression in Arc induced by ghrelin with and without manganese (Mn).

Figure 4. Photomicrographs of the c-fos expression of the Arc in a) PBS without manganese (Mn), b) ghrelin without Mn, c) PBS with Mn, d) ghrelin with Mn.

Fig 3

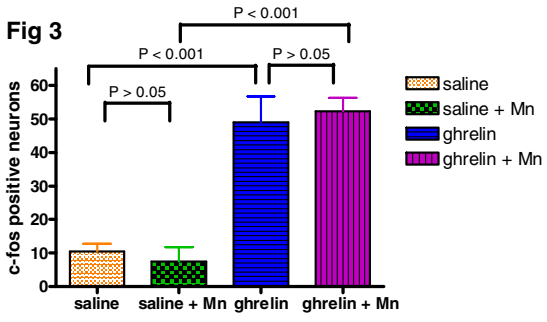
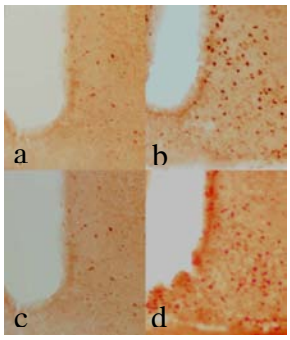


Fig 4



Conclusions: MEMRI enhancement can be modulated by the gut-peptides, ghrelin and PYY_{3-36}, in a region-specific manner. Systemic administration of manganese with our technique does not significantly change the c-fos expression of the murine brain. This also suggests that MEMRI is an invaluable non-invasive tool to evaluate hypothalamic activation *in vivo*.

References: 1. Morita, H. et al. Neurosci Lett 326:101-104, 2002
2. Aoki, I. et al. Magn Reson Med 48:927-933, 2002