Manganese-Enhanced MRI of Appetite Centres in ob/ob Mice

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Introduction

Objective measurement of neuronal activity in relation to appetite control currently uses methods that are either highly invasive and/or fatal, such as immunohistochemistry for the early-expressed neuronal activation protein c-Fos. To measure changes over time using such methods requires parallel study groups culled at the different times. A non-invasive strategy such as using Manganese-Enhanced MRI[1] (MEMRI) to analyze the same individuals over a time course would 1) reduce the number of animals used, 2) reduce experimental variation, and 3) possibly allow the direct study of humans.

We recently determined that MEMRI (with Mn^{2+} administered by intravenous infusion) could, in terms of magnitude at several appetite centres in the brain, differentiate between the neuronal activities of mice that had been fed normally or fasted overnight[2]. The aim of this study was to extend the above feeding-fasting experiment to leptin-deficient (*ob/ob*) mutant mice. The *ob/ob* phenotype includes obesity, hyperinsulinaemia, hyperlipidaemia and defective thermoregulation. This mouse model is used extensively to probe the mechanisms of mammalian hunger control, so it was essential to determine whether MEMRI could perform well in this situation.

Materials and Methods

All experiments complied with the Animal (Scientific Procedures) Act 1986 (UK). Mice were 11-15 wk old homozygous C57BL/6OlaHsd-*Lep*^{ob} males (Harlan, UK) referred to here as *ob*. Isoflurane-anaesthetised mice were implanted with a tail vein cannula and placed in a 25 mm internal diameter quadrature head coil (Magnetic Resonance Laboratories, Oxford, UK). The cannulated mouse, coil and a saline phantom were placed at the centre of a 9.4 T horizontal bore MR scanner (Varian, Palo Alto, CA, USA). Sixty-six image stacks of the brain were acquired consecutively over a period of just over 2 hours (1 m 57 s per acquisition) using a spin-echo multi-slice T1-weighted sequence with TR=600 ms, TE=10 ms, a 256×192 voxel matrix (zero-filled to 256×256), 25 mm × 25 mm field of view, single averages, and 10 transverse slices of 1 mm thickness each. After three baseline acquisitions, 62.3 mM $MnCl_2$ was infused via the cannula by a syringe pump (PHD 2000, Harvard Apparatus, MA, USA) for around 40 mins (exact time proportional to body weight) at a rate of 0.2ml/h. The signal intensity (SI) of different brain structures was measured by drawing regions of interest (ROI) with the image processing software Image J (version 1.33, NIH, USA). Corrections were made for machine, infusion and inter-animal variation by normalization to phantom SI, SI of 4th ventricle/choroids plexus boundary, and SI of the baseline scans respectively.

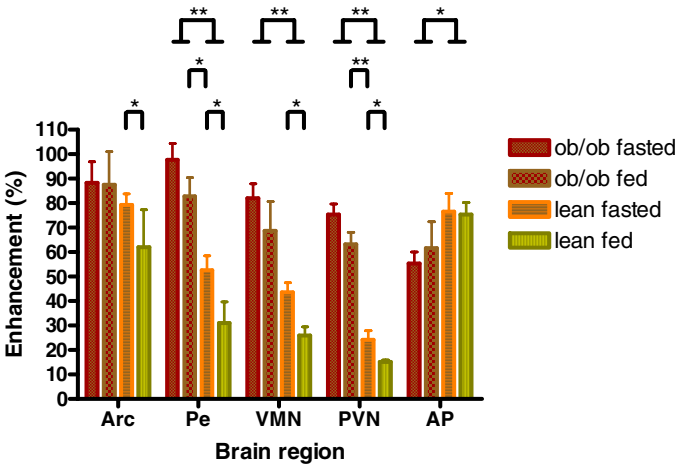

Figure: graph of steady-state enhancement in selected brain regions. Lean mouse data is from [2]. Error bars are standard deviations. Significant differences (* p<0.05, ** p<0.001) between groups are indicated by the starred markers for fed/fasted (lower row) fed ob/fasted lean (middle row) and lean/ob (upper row) comparisons. Arc=arcuate nucleus, Pe=periventricular nucleus, VMN=ventromedial nucleus, PVN=paraventricular nucleus, AP=anterior lobe of the pituitary gland.

Results

Mice in groups of six were either fasted for 12-18 hrs overnight or fed normally before being processed and imaged by MEMRI. Enhancement (% SI increase over baseline) reached a steady-state during final 15 image stacks as reported previously[2].

The results of the MEMRI analysis are presented in the Figure. The first feature to note is that fasting state does not regulate the enhancement of the AP in *ob* mice, providing evidence that MEMRI behaves similarly in the *ob* and wild-type environments.

No significant differences in enhancement were found between fed and fasted groups of *ob*. When fed and fasted groups were combined, despite the inevitable increase in variance, significant differences in enhancement were found between lean and *ob* groups for most brain regions (Pe, VMN and PVN p<0.001, AP p<0.05). In all cases apart from the AP, enhancement was higher in *ob* mice. An interesting comparison is that between fasted lean and fed *ob*. This test considers whether the enhancement due to fasting (an acute, short-term situation) is of a similar magnitude to that generated as a result of starvation (a chronic, long-term situation that *ob* mice represent). Again, enhancement was higher in *ob* mice in all brain regions apart from the AP where it was lower. However, these differences were only significant in the cases of the Pe and PVN (p<0.05).

Discussion

In this study, as measured by MEMRI, no significant differences were found in the neuronal activity of appetite-regulating brain regions between fed and fasted groups of *ob* mice. This is probably a consequence of the acute feeding state effect being saturated by the chronic starvation effect. However, inconsiderate of feeding state, *ob* mice were found to have significantly higher levels of activity than lean in the Pe, VMN and PVN. Even fasted lean mice had lower activities in their appetite centres than fed *ob* (though in this case the difference was not significant for the VMN). These results are expected since *ob* mice are innately starved, so the activity of their appetite centers should be higher than that of lean mice.

There is some agreement between these results and those from c-Fos immunohistochemistry[3], which suggest that feeding state has no significant effect on *ob* neuronal activity. However, the c-Fos data indicates that *ob* mice merely have moderately raised levels of activity in their appetite centres compared to lean. Thus MEMRI and c-Fos results are consistent with respect to acute effects, but diverge with respect to chronic effects. This discrepancy needs to be resolved, and may reveal important information about the relationship between c-Fos levels and neuronal activity, which currently is not known beyond a few hours[4].

In conclusion, *in vivo* MEMRI confirms previous *in vitro* c-Fos results for acute fasting in the *ob* environment, though there are discrepancies with results of chronic states.

References

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