A fMRI study to decipher the regional effects of an intraperitoneal glucose dose in the fasted rat model

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INTRODUCTION
All neurons consume glucose to fulfil their metabolic needs. However, specialised ‘glucosensing’ neurones exist that regulate membrane potential and firing rate according to ambient glucose levels1. Such neurones are generally located in brain areas involved in neuroendocrine function, nutrient metabolism and energy homeostasis, receiving direct and indirect neural input from the periphery and brain areas conveying sensory information, reward properties and blood levels of nutrients1. The neurones may either exhibit increased activity (glucose-excited, GE) or decreased activity (glucose-inhibited, GI) as ambient glucose levels rise1. BOLD-MRI has been used to provide an indirect measure of neuronal activity in the hypothalamus following non-oral glucose administration in both rats2 and humans3. In this study, we have assessed the effect of an intraperitoneal (i.p.) glucose dose on the BOLD-MRI signal throughout the brain of a fasted rat model.

METHODS
Animals and Treatment: Sprague-Dawley rats (n=10, 252.7g ± 8.3g, Harlan UK) were fasted overnight (~20h). Anaesthesia was induced with 3% isoflurane in air/O2 (70:30) mix and an i.p. cannula implanted. The head was secured in a holder and placed within a volume coil and MRI performed on a 7T MRI system with anaesthesia maintained at 1-1.5% isoflurane. A structural scan was obtained using a fast spin-echo sequence: TR, 4s; TE, 60ms; 24 contiguous 1mm thick coronal slices and 4 averages. BOLD-MRI was also performed using a multi-echo gradient echo sequence: TR, 125.18ms; TE, 5, 10 and 15ms (mean echo was analysed); the same slice selection as before; 32s per scan acquisition time. 40 scans were collected prior to, and 140 after, an i.p. injection of isotonic glucose (1g/kg, 20ml/kg water, n=5) or 0.9% isotonic saline (n=5). Physiological parameters (temperature, pulse, oxygen saturation and respiration) were monitored throughout. After MRI, animals were recovered for 40mins prior to sodium pentobarbital overdose, and terminal cardiac blood samples collected for glucose measurements.

Image Analysis: Data was analysed by SPM5 software. BOLD-MRI images were movement corrected, non-brain tissue masked and images normalised to a rat brain template, prior to Gaussian smoothing (2x in-plane resolution). The random effects general linear model (2nd level analysis) was applied to test for changes in the BOLD signal between pre- and post-glucose images in the time-series. SPM(t) distribution was thresholded at p<0.001 (uncorrected for multiple comparisons).

RESULTS AND DISCUSSIONS
Plasma glucose concentrations was significantly higher after injection of glucose (223.2±53.4mg/dl, mean±sd) compared to saline (147.9±22.16mg/dl, P<0.05). Significant widespread increases in the BOLD-MRI signal after glucose administration were observed in the cerebellum, brainstem, other hindbrain and midbrain regions, hippocampus, hypothalamus, thalamus and striatum (Figure 1). In contrast, less extensive changes were observed following saline, with only small increases in the posterior cortex and (unilateral) hippocampus (data not shown). Both saline and glucose-treated animals showed significant BOLD-contrast decreases in the prefrontal cortex and olfactory regions, possibly related to i.p. volume administration.

Significant activations were observed in the hypothalamic and hippocampal areas. The activations in the former are consistent with the known presence of glucosensing neurones in the ventromedial nucleus containing higher proportions of GE neurones4. Likewise, glucosensing neurones are also present in the hippocampus, the neurones becoming more depolarised, i.e. excitable with increasing glucose concentrations5. However, the changes observed following glucose dosing may also result from insulin action in the brain: insulinemia, arising from glucose-stimulated release of insulin from pancreatic beta cells. We did not observe a transient decrease in activity in the hypothalamus previously reported3,4 following i.p. glucose administration. Our methodology may be insensitive to this transient small decrease but sensitive to changes occurring throughout the brain. Further studies are needed to determine the source of the changes in the BOLD-MRI signal following i.p. glucose administration.

CONCLUSION
We have shown the ability to non-invasively assess changes in the brain following a glucose dose, suggesting the possibly use of this technique to determine the functional role of various nutrients under different physiological states.

REFERENCES