FMRI of The Hypothalamus Following Glucose Administration in A Rat Model: Implications for Obesity and Diabetes Research

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Introduction: A recent fMRI study (1), demonstated a delayed and attenuated acute transient inhibitory hypothalamic fMRI response following glucose ingestion in obese non-diabetic humans, as compared with lean non-diabetics. To further study the mechanism and specificity of this observed response by the exogenous administration of different nutrients and medications, a Sprague-Dawley (SD) rat model was developed, the details of which are mentioned below.

Methods: Six healthy male Sprague-Dawley (SD) rats (age: 21.8 ± 13.2 wks; wt: 378.± 82.5 gms), fasted overnight (12 hrs) prior to the fMRI scan (1.9 T MRI scanner, GE/Elscint Ltd., Haifa, Israel), were anesthetized by an intramuscular injection of rat cocktail (6 cc ketamine (60 mg/ml) and 4 cc rompun (40 mg/ml) for a total of 10 cc) into the thigh muscle which was re-administered again after 60 minutes (0.1–0.2 cc/250 gms body wt.). Each rat was placed supine on a restraining device and a butterfly needle introduced into the peritoneal cavity for glucose administration during the fMRI scan (0.72 gms/kg body weight). Its free end was connected to a 2cc syringe containing 25% glucose solution (75 g of carbonated D-dextrose dissolved in 296 ml of orange flavored water)(SIP Orange Glucose Tolerance Beverage 75, Criterion Sciences, Division of Cornwell Corp., Riverdale, NJ).

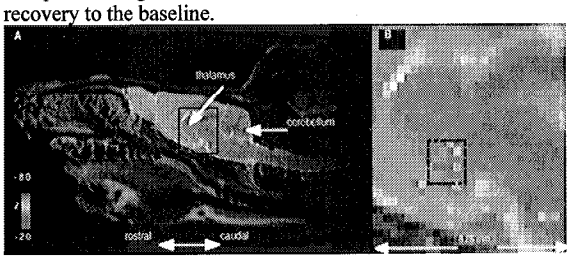
A FSE coronal scan (TR/TE=3000ms/90ms; 2.5 mm slice thickness; 0.3 mm x 0.3 mm pixel size) was initially performed to accurately identify the hypothalamus. The corresponding sagittal slice was then continuously imaged by a conventional T_2*-weighted GE pulse sequence (TR/TE/θ =60/40/20^0; 2.5 mm thickness; 0.3 mm x 0.3 mm pixel size; 235 averages). Ten minutes after baseline fMRI scanning (40 images), glucose solution (0.8 ml-1.5 ml; based on body wt.) was injected intraperitoneally followed by the continuation of scanning for another 50 minutes (195 images).

Data processing was performed on a SUN Ultra30 workstation using a MEDX package (Sensor Systems, Inc., USA) and in-house programs written in C and MATLAB (The Mathworks, Natick, MA). The boundary of the rat hypothalamus was determined by previous criteria (2). All the images obtained for each rat were realigned to a reference image and corrected for motion using the AIR program (3). The images obtained after glucose administration were divided into 10 time frames with each containing 20 images. The last group had only 15 images. Multiple comparisons between the baseline and each time frame after glucose administration were performed with group *t*-tests to determine significant changes in the mean MR signal intensity induced by glucose administration. The *t* values for each pixel were then transformed to z scores using MEDX. To afford a $p < 0.05$ level of statistical significance of the detected signal changes a pixel-clustering size of 3 and a *z*-threshold of $|z| > 2.0$ were chosen (4). Finally, the resultant statistical parametric images (color coded) were overlaid on the corresponding FSE anatomical image acquired with the same field of view and image matrix sizes as the T_2^* images.

Two quantitative indices were used to analyze the observed fMRI response: mean inhibition I_{mean}, expressed as the percentage change of MRI signal over the period of time after intraperitoneal glucose administration relative to that of the baseline, and T_d, measured as the time lag from the beginning of intraperitoneal glucose administration to the time at which the fMRI signal reached its maximal inhibition.

For the specifically selected ROI (rat hypothalamus), the time course of the MRI signal intensity, averaged on a pixel by pixel basis, was generated by normalizing the averaged value at each image time to that of the first image of the baseline. To reduce the influence of the MRI signal fluctuation on the calculations, a segmented polynomial curve fit (n = 5, in a least-squares sense) was conducted to fit the data of the time course of the ROI. The I_{max} (maximum magnitude of inhibition) was measured from the fitted curve. I_{mean} was then calculated by dividing the change in the MRI signal during a time window of 14 minutes centered at I_{max} over the baseline.

Results: An acute transient decrease in the fMRI signal intensity was observed in all the rats within 12-16 mins after intraperitoneal glucose administration, which was followed by a recovery to the baseline.



Figures 1(A) and 1(B) above illustrate a typical example of the fMRI response observed in a SD rat following intraperitoneal glucose administration.

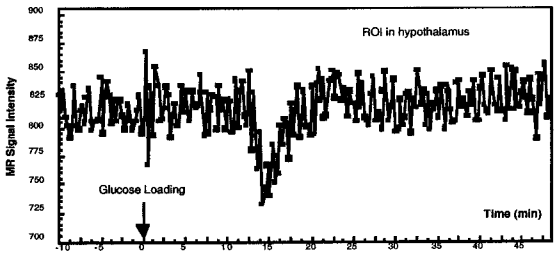


Figure 2 above shows the time course of the MRI signal intensity of the activation area within the hypothalamic region depicted in Figure 1(B). For this rat, the acute transient decrease in fMRI signal intensity was observed at 12 mins following intraperitoneal glucose injection and reached a maximum at approximately 14 mins before returning to the baseline after 20 mins. The quantitative analysis of the fMRI responses observed in all the 6 male SD rats following intraperitoneal glucose administration showed a mean I_{mean} of 3.4 ± 0.4% and T_d of 16.4 ± 1.0 mins.

Conclusions: The SD rat is an excellent model for fMRI studies in obesity and diabetes mellitus involving the administration of exogeneous nutrients and medications that are impractical to perform in humans.

References:

(1) Matsuda M, Liu Y, Mahankali S, Pu Y, Mahankali A, Wang J, DeFronzo RA, Fox PT, Gao J-H. Altered hypothalamic function in response to glucose ingestion in obese humans. Diabetes 48 (9):1801-1806; 1999.

(2) Toga AW, Santori EM, Hazani R, Ambach K. A 3D digital map of rat brain. Brain Research Bulletin 1995; 38:77-85.

(3) Woods RP, Cherry SR, Mazziotta JC. Rapid automated algorithm for aligning and reslicing PET images. J Comput Assisted Tomogr 1992; 16:620-633.

(4) Xiong J, Gao J-H, Lancaster JL, Fox PT. Clustered pixels analysis for functional MRI activation studies in human brain. Human Brain Mapping 1995; 3:210-225.