Increased plasma sodium induced neural activations in the hypothalamus: an fMRI study

M. Dashti[1,2], J. Williams[1], M. Geso[2], G. Egan[1], B. Purcell[1], P. Burns[1], R. Weisinger[1]

[1]Neuroimaging and Neuroinformatics, The Howard Florey Institute of Experimental Physiology and Medicine, Melbourne, Victoria, Australia, [2]Radiation Sciences, Royal Melbourne Institute of Technology (RMIT) University, Bundoora, Victoria, Australia

Introduction: Maintenance of body fluid and sodium homeostasis is crucial to life. An understanding of what regions of the brain are functionally involved in body fluid homeostasis is important in reaching an understanding of diseases affecting ionic balance. Functional MRI (fMRI) has recently been used to demonstrate neural activity associated with thirst stimuli in humans (1). In this study, we utilised EPI based-fMRI to determine the brain regions influenced by changes in blood sodium concentration.

Methods: Four healthy male Sprague-Dawley rats (371±49 grams) were prepared with an indwelling venous cannula for saline infusion 3 – 4 days prior to the fMRI experiment. On the day of the experiment, each rat was anaesthetised with Isoflurane, and placed prone in a specially designed plastic cradle (minimising head movement), and imaged using RARE and EPI pulse sequences (Bruker Biospec 47/30). RARE axial images were acquired using the following parameters: TR/TE=3481ms/67.5ms, FOV = 60 mm, MTX = 256 x 256, slice thickness and gap = 1 mm. A subset of 9 axial slices from the RARE images were then acquired in the EPI experiment (960 volumes), using the parameters: TR/TE=3745ms/9.9ms, MTX = 64 x 64, EPI_trim = 67 µsec, and no segmentation of the EPI echo train. The EPI scan was divided into three main experimental intervals for data analysis: 0 - 10 minutes (160 volumes) of normal saline infusion; 40 minutes (640 volumes) of hypertonic saline (4 M NaCl, 1 ml/hr) infusion; and 10 minutes (160 volumes) of no infusion. Cerebral anatomy from the RARE images was acquired in registration with the EPI scans to ensure accurate anatomical localisation of neuronal activity. Data processing and analysis was performed on a UNIX workstation using SPM99 (Wellcome Institute, London) for analysis and MRIcro for overlays. The SPM99 analysis model divided the entire 60 minutes of EPI data into six successive intervals of 10 minutes each, with each 10 minute interval analysed as a conjunction of ten successive one minute responses. Multiple comparisons between the baseline and each time interval of hypertonic saline infusion were performed with group *t*-tests to determine significant changes in the mean MR signal intensity. This was correlated against each voxel to determine regions of activation (Corrected $P<0.05$). Finally, the resultant SPM99 activation maps were overlaid on the corresponding RARE T_2-weighted anatomical images for each time interval. The boundaries of sites of neuronal activation were determined by comparing the RARE images to corresponding histological slices from the Paxinos and Watson rat atlas (2).

Behavioural measurements (water intake) using the same infusion protocol of hypertonic saline were performed 24-48 hours before or after each MRI session. This allows for comparison of the fMRI responses to the behavioural measurements and to ensure functional behavioural modification occurred in each animal with this infusion protocol.

Results: All rats demonstrated drinking within 30 minutes of starting an IV infusion of the hypertonic saline. The fMRI experiments showed a significant increase in BOLD signal in all experiments (n=4), particularly in the thalamus and the hypothalamus (see arrows in Figure-1). Z-scores for the activations measured for the period 30 to 40 minutes of hypertonic saline infusion are presented in Table 1.

Rat ID	Hypothalamus
A	3.5
B	3.6
C	3.6
D	3.4

Table-1: Z scores at ~35miutes of infusion.

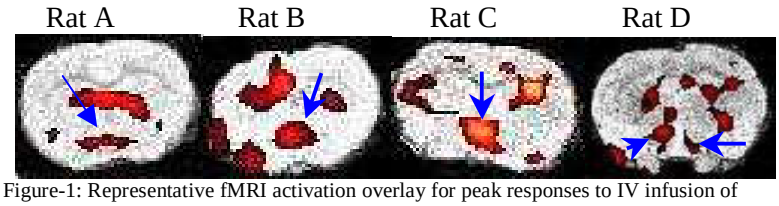

Figure-1: Representative fMRI activation overlay for peak responses to IV infusion of hypertonic saline at 30 –40 minutes of infusion in each rat. Arrow(s) indicate hypothalamus.

Conclusion: We have demonstrated that IV infusion of hypertonic saline consistently produces an increase in neural activity in specific regions of the rat brain under conditions of Isoflurane anaesthesia. These regions could be clearly identified using the T_2-weighted images acquired before the fMRI scans for each appropriate slice in the EPI volume. Dividing the entire fMRI period up into defined time series allowed increased sensitivity to activations produced by a slowly changing physiological variable, in this case, plasma [sodium]. The brain areas that showed activation are known to be involved in the regulation of thirst as shown in experiments utilising lesion and c-Fos techniques (3). This confirms the efficacy of using fMRI to study brain regions involved in physiological regulation of body fluid homeostasis.

References:

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