BOLD fMRI Response of the Rat Brain to Hyperosmotic Saline Infusion

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Target Audience: Neuroscientists, Translational Scientists, Researchers studying animal models

Purpose: Regulation of systemic osmolality is an essential function in mammals. Mammals have developed homeostatic responses such as increased thirst for water, decreased salt appetite, release of vasopressin for increased retention of water in the kidneys, and increased rate of natriuresis in response to extracellular hyperosmolality[1]. It has also been shown that hyperosmolality can increase sympathetic nerve activity in the brain [1,3]. Osmosensitive neuron located in areas of the brain that lack a blood brain barrier such as the subfornical organ (SFO), and organum vasculosum laminae terminalis (OVLT) within the forebrain can detect small changes in extracellular osmolality [1,3]. Electrophysiological, histological, and lesion studies suggest that hyperosmolality activates neurons in the OVLT that project to the hypothalamic paraventricular nucleus (PVN) which is involved with water drinking, pituitary hormone release, and sympathetic activation[3-4]. Areas of increased sympathetic activation are of interest because elevated sympathetic activity is present in most forms of hypertension [2].

In this study we will use BOLD fMRI imaging to see areas of brain activation that occur in response to an infusion of alternating isotonic and hypertonic saline into the right internal carotid of a rat. This application of BOLD fMRI is able to non-invasively demonstrate areas of brain activation in response to hypertonicity that may be previously unknown. Different activation patterns between control and hypertensive animals may further elucidate additional targets for the treatment of salt-sensitive forms of hypertension.

Methods: Three trials from two adult male Sprague Dawley rats (350-500g) were studied. Rats were initially anesthetized with 3% isoflurane and orally intubated for mechanical ventilation. In each rat, the right common carotid artery was tied off and thinned PE50 tubing was inserted into the common carotid artery 0.5cm below the carotid bifurcation. The tubing was advanced up the internal carotid 1cm toward the brain. A suture was tied around the internal carotid and to prevent backflow activation of baroreceptors in the carotid sinus. Then the animal was secured in head holder consisting of ear and tooth bars and the level of isoflurane was decreased to 1.2%. BOLD MRI was acquired on a 7-Tesla/40cm magnet (Biospec Bruker) using single shot gradient-echo, echo-planar imaging with TR= 3s, TE=21ms, spectral width=150kHz, matrix=96x96 reconstructed to 128x128, FOV=23x23mm, 1mm slice thickness. Each scan had 2 minutes of isotonic saline infusion (0.9% or 0.3 osmol/kg), alternating with 2 minutes of hypertonic saline (4.5% or 1.5osmol/kg) repeated for a total scan of 12 minutes. Infusion rate was set for 0.1ml/minute.

Results: Figure 1 shows a cross-correlation map superimposed on the average EPI image from three trials in a rat. The slices are positioned from bregma -0.3mm to bregma -2.3 mm. There was a significant difference (p=0.029) between the average percent increase from baseline for isotonic saline (-0.85%) and hypertonic saline (13.79%).

Discussion: Activation of the anterior medial hypothalamus was present with a significant increase in signal intensity found in response to hyperosmotic saline infusion challenge. The areas of activation found follow those found by previous studies on osmoregulatory circuits. Activation in this region is of great interest due to its role in increased sympathetic activity.

Conclusion: This study has shown that areas of interest found via electrophysiology, histological, and lesion studies can also be identified using fMRI, allowing non-invasive longitudinal studies. Future studies including electrophysiological recordings to confirm recruitment of sympathetic activity and inclusion of hypertensive rat models are warranted.

References: 1. Bourque C. (2008) *Neuro* **9**, 519-531. **2.** Guyenet P.G. (2006) *Neuro* **7**, 335-346. **3.** Shi P. et al. (2008) *J Physiol* **586.21**, 5231-5245. **4.** Shi P. et al. (2007) *Am J Physiol Regul Intergr Comp Physiol* **293**, R2279-R2289. **5.** Egan G. et al. (2003) *PNAS* **100.25**, 15241-15246.

Figure 1: A cross correlation map superimposed on the average EPI image from a single rat in response to hypertonic infusion. Activation is present in the anterior medial hypothalamus in which osmosensitive neurons are present.