BOLD fMRI Response of the Rat Brain to Bolus Injection of Hypertonic Saline

Bianca Gonzales Cerqueira[1], Qiang Shen[1], Fang Du[1], Glenn M. Toney[2], and Timothy Q. Duong[1]

[1]*Research Imaging Institute, University of Texas Health Science Center at San Antonio, San Antonio, TX, United States,* [2]*Department of Physiology, University of Texas Health Science Center at San Antonio, San Antonio, TX, United States*

Introduction: Homeostatic regulation of systemic osmolality is an essential function in mammals [1]. Changes in osmolality modulate sympathetic nerve activity in the brain [1,3]. Osmosensitive neurones (such as the subfornical organ (SFO) and organum vasculosum laminae terminalis (OVLT) within the forebrain) are located in areas of the brain that lack a blood brain barrier and they can detect small changes in extracellular osmolality [1,3]. Electrophysiological, histological, and lesion studies demonstrate that hyperosmolality activates neurons in the OVLT that project to the hypothalamic paraventricular nucleus (PVN). The latter brain region is critically involved with water drinking, pituitary hormone release, and sympathetic activation [3,4]. Areas responsible for increasing sympathetic activity are of keen interest because elevated sympathetic outflow is an important mechanism in many forms of salt-sensitive hypertension [2].

In this study, we used BOLD fMRI to map brain areas activated immediately following a bolus injection of 200µl hypertonic saline into the right internal carotid of rats. Our ultimate goal is to identify novel areas of activation and to determine if such sites can be validated as new neuronal targets for treatment of salt-sensitive forms of hypertension.

Materials/Methods: Adult male Sprague Dawley rats (n=4, 250-415g) were initially anesthetized with 3% isoflurane and orally intubated for mechanical ventilation. The right internal carotid artery was catheterized above the carotid bifurcation to prevent stimulation of the carotid body. Each animal was secured in a head holder consisting of ear and tooth bars. Thereafter, the level of isoflurane was decreased to 1.2%. Each rat was paralyzed with pancuronium bromide (3mg/kg first dose, 1mg/kg/hr, ip). BOLD fMRI was acquired on a 7-Tesla/40cm magnet (Biospec Bruker) using single shot gradient-echo, EPI with TR= 3s, TE=21ms, spectral width=150kHz, matrix=96x96 reconstructed to 128x128, FOV=23x23mm, 17 1mm slice thickness, total acquisition time of 10 minutes for each trial. A warmed (body temperature) bolus of 200µl of isotonic (0.3 osmol•kg^{-1}) or hypertonic (1.5 osmol•kg^{-1}) saline was injected over 3-5 seconds at 2 and 7 min into acquisition. Two trials of isotonic and three trials of hypertonic injections were made on each animal with 15 min breaks in between. Images were corrected for motion and drift. Cross-correlation analysis was performed for BOLD fMRI scans.

Results: As controls, isotonic injection produced no significant nor consistent activations in the brain (data not shown). **Figure 1** shows a fMRI map superimposed on the average EPI image from one rat injected with hypertonic saline and the corresponding time course of activated pixels. Cingulate cortex, somatosensory cortex, fornix/subfornical organ, periaqueductal gray and hypothalamic paraventricular nucleus were activated in all animals (100% incidence). The insular cortex, median preoptic nucleus, and field CA3 of the hippocampus were activated in 75% of the animals. The OVLT was activated in 50% of the animals.

Discussion and Conclusion: This study reports the use of MRI to non-invasively map osmoregulatory circuits. A novel area of activation was found in field CA3 of the hippocampus. Established areas of activation consistent with many structures within the osmoregulatory circuits reported in electrophysiological, histological, and lesion studies were also found. For example, the OVLT projects to the cingulate cortex, hypothalamic paraventricular nucleus, median preoptic nucleus, supraoptic nucleus, and thalamus [1]. It has been suggested that the sensation of thirst originates in the insular cortex while the cingulate cortex is responsible for motivation of behavioral responses such as drinking water [1]. The periaqueductal gray may also be responsible for behavioral responses to thirst [1]. The fornix/subfornical organ has been shown to be osmosensitive so activation is expected in that region[1]. Activation of the hypothalamic paraventricular nucleus is of great interest due to its role in increased sympathetic activity. Future studies are warranted to use electrophysiological recordings to confirm both established and novel areas of activation associated with osmoregulation. Our ultimate goal is to identify and validate new sites for targeting treatment of salt-sensitive forms of hypertension.

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.

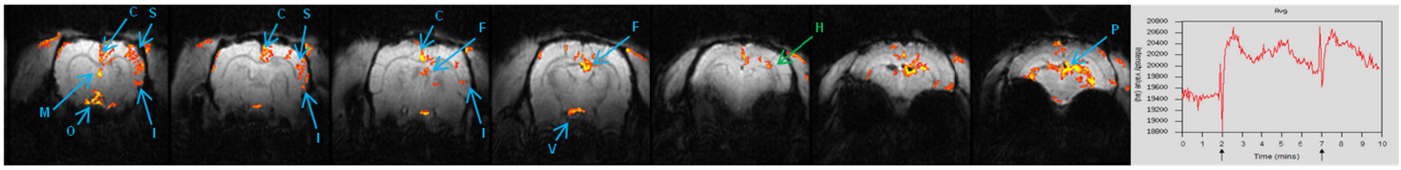

Figure 1: Activation map on EPI image from one rat injected with hypertonic saline and the signal time course from all activated pixels in the brain. Injection of hypertonic saline occurred at time points 2 and 7 mins (arrows). Labels: cingulate cortex (C), somatosensory cortex (S), fornix/subfornical organ (F), periaqueductal gray (P), hypothalamic paraventricular nucleus (V), median preoptic nucleus (M), OVLT (O), CA3 field of hippocampus (H).