

# Perfusion fMRI Reveals Cerebral Blood Flow Pattern under Psychological Stress

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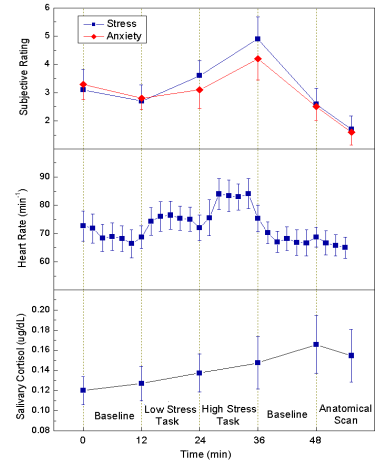
**Introduction** Despite the prevalence of stress in everyday life and its wide influences on happiness, health and cognition, little is known about how the brain activates when people experience stress. Arterial spin labeled (ASL) perfusion MRI directly measures cerebral blood flow (CBF) by using arterial blood water as an endogenous contrast agent. With excellent reproducibility over long term time periods (1) and minimal sensitivity to magnetic field inhomogeneity effects (2), perfusion fMRI is ideally suited for imaging sustained behavioral state that involves the function of deep brain structures, such as stress. Perfusion fMRI also allows ecological paradigms to be used in the MR scanner to induce “natural” stress, owing to its reduced scanner noise level and sensitivity to subject motion. Here we report the first attempt using ASL to directly map the neural correlates of psychological stress induced using mental arithmetic tasks.

**Materials and Methods** Ten subjects (age  $25.5 \pm 2.5$  yrs, 3 female) participated in the stress experiment on a 3.0T Siemens Trio MR scanner with a standard volume head coil. A continuous ASL sequence which has been optimized for 3.0T was used for perfusion MRI scans (3), with the parameters: FOV=22cm, 64x64 matrix, TR/TE=4000/17ms, delay time=1000ms, gradient-echo EPI acquisition, 14 axial slices with 6 mm thickness and 1.5 mm gap. The scanning protocol consisted of 4 perfusion fMRI scans (120acq/8min each) and a MPRAGE anatomical scan (6min) at the end. The first and last perfusion fMRI scans were baseline conditions without task. During the second and third perfusion fMRI scans, subjects were instructed to perform counting backward from 1000 (low stress) and serial subtraction of 13 from a 4-digit number (high stress) respectively. During the high stress task, the subjects were also prompted for faster performance and were required to restart the task if an error occurred. Self report of stress and anxiety level (on the scale of 1 to 9) as well as saliva samples were collected right after the subjects entered the MR scanner and after each MR scan. Subjects were also required to report the level of effort, frustration and task difficulty after the low and high stress tasks. Throughout the experiment, heart rate was recorded every two minutes based on a pulse-oxymetry reading.

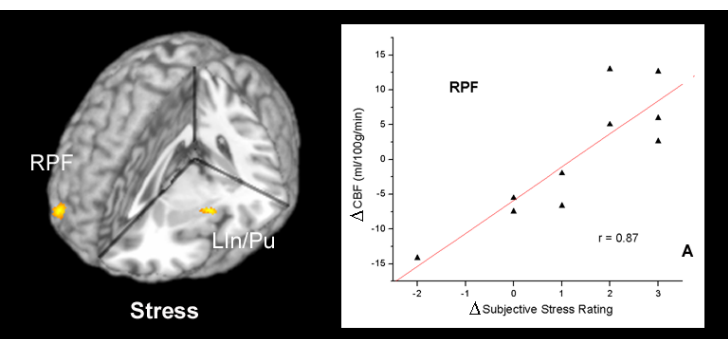
Perfusion fMRI data were analyzed offline using the VoxBo and SPM99 software packages. MR image series were first realigned to correct for head movements, co-registered with each subject’s anatomical MRI, and smoothed in space with a 3D 12mm FWHM (Full Width at Half Maximum) Gaussian kernel. Perfusion weighted image series were generated by pair-wise subtraction of the label and control images, followed by conversion to absolute CBF image series based on a single compartment CASL perfusion model (3). Voxel-wise analyses of the CBF data were conducted in each subject, utilizing a general linear model (GLM) including the global time course as a covariate. Two contrasts were defined in the GLM analysis, namely the CBF difference between the two stress tasks (high – low stress) and the CBF difference between the two baseline conditions (2nd – 1st baseline). Individual contrast images were normalized into a canonical space (Montreal Neurological Institute), and entered into a random effect group analysis. One-sample t-tests were used to obtain the activation pattern for the two defined contrasts. Linear regression analyses were carried out on these normalized individual maps to obtain the activation pattern correlated with perceived stress and other measurements, using differences in each of the behavioral and physiological measurements between the high and low stress tasks as the independent variable. Areas of significant activation were identified at the cluster level for the  $P$  value smaller than 0.001 (uncorrected) and the cluster extent size larger than 30 voxels ( $2 \times 2 \times 2 \text{mm}^3$ ), resulting in a cluster corrected threshold of  $p < 0.05$  in SPM99.

**Results** Our records of subjects’ stress, emotion and physiological responses demonstrated that the stress elicitation paradigm was successful. Average self report of stress ( $p=0.003$ ) and anxiety ( $p<0.001$ ) levels as well as the heart rate ( $p<0.001$ ) increased from the low stress task to the high stress task, and decreased during the relaxation period (Fig. 1). Salivary cortisol, a stress related hormone, steadily increased across the experiment and reached its peak 10 minutes after the end of the high stress task ( $p=0.02$ ), consistent with the expected time lag between peripheral cortisol and behavioral measures. Subjects’ ratings of task difficulty ( $p<0.001$ ), effort required ( $p=0.001$ ) and frustration ( $p=0.002$ ) were significantly elevated in the high stress condition relative to the low stress condition. In addition, we found that perceived stress level was significantly correlated with perceived anxiety level across subjects ( $r=0.78$ ,  $p=0.007$ ).

Regression analyses showed a positive correlation between the changes in CBF and subjective stress rating between the high and low stress tasks in the ventrolateral right prefrontal cortex (RPF) and left insula/putamen (LIn/Pu) area (Fig. 2), which have been linked with the processing of certain forms of negative affect, especially fear and disgust. A further regression analysis revealed a strong correlation between changes in baseline CBF and subjective stress rating during stress tasks in the right prefrontal cortex and anterior cingulate cortex, which are important regions involved in emotion and vigilance/attention. The scatter plot in Fig. 2 shows that the serial subtraction task yielded a greater CBF increment in right prefrontal cortex in subjects who reported larger amount of stress elevation, and this activity even lasted after the task was completed. Paralleling behavioral data showing correlation of perceived stress and anxiety, similar pattern of brain activation (especially right prefrontal activation) was observed when self report of anxiety was used as the predictor for regression analyses.



**Fig. 1** Average subjective ratings of stress and anxiety, heart rate and salivary cortisol level during the time course of the stress experiment.



**Fig. 2** 3D rendering of regression analysis results (CBF vs. self report of stress change from low to high stress task). Scatter plot shows correlation in RPF.

## Reference

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