INTRODUCTION: An important application of fMRI is to provide a marker for treatment outcome. Typically, fMRI sessions were conducted before and after medications, and the amplitude of fMRI signals in the hypothesized brain regions was compared to determine whether the medication has enhanced or suppressed neural activity. A potential problem with this approach is that other consequences of the medication, including global changes in physiology, are not considered in comparing the fMRI signals. Many drugs alter brain vasculature in spatially non-specific manner, which could influence the amplitude of fMRI signal because fMRI is based on vascular responses and is known to be modulated by a number of baseline physiologic parameters (e.g. resting blood oxygenation) independent of neural activity (1). The modulation effect can be conceptually appreciated by considering that fMRI signal is based on changes in blood oxygenation ($Y_v$) from resting to activated conditions, therefore a lower resting $Y_v$ would mean that it has more room to go up, thereby a greater fMRI signal for the same neural activity (2). Here we demonstrated that hydrocortisone, a stress and corticosteroid hormone that are used to treat asthma but could cause memory decline (3), reduces resting venous blood oxygenation globally and that normalized fMRI signal after accounting for this global change allowed the detection of hippocampal alterations after merely three days of medication.

METHODS: Experiments: Fifteen young, healthy subjects participated in a randomized, double-blind, placebo-controlled trial of hydrocortisone for effect on brain function. Beginning 2 days prior to the imaging, participants began taking four tablets containing hydrocortisone (20 mg) or placebo at 0900 hours and 2100 hours (160 mg/day) with the last dose at approximately 0900 hours on the day of the imaging. MRI was performed at approximately 1300 hours on a 3T scanner (Philips). Each subject participated in both a hydrocortisone and a placebo trial and a minimum gap of 21 days were used to ensure wash-out. The MRI session consisted of five fMRI runs with a total duration of 30 min. The fMRI used a novelty detection (episodic memory) task in which the subject viewed familiar or novel pictures in an event-related design (200 trials in total). Standard BOLD imaging parameters were used: TR/TE/flip angle=1500ms/30ms/70°, 30 axial slices, voxel size 3.44x3.44x5 mm$^3$. In addition, global resting blood oxygenation was determined using a recently developed T2- Relaxation-Under Spin-Tagging (TRUST) technique, which uses spin labeling principle to isolate pure venous blood signal and then estimates its T2 (4). TRUST imaging parameters were: TR/TE/T1=8000ms/7ms/1200ms, voxel size 3.44x3.44x5 mm$^3$, duration 4 min; the global venous oxygenation was measured from the sagittal sinus which is the main draining vein in the brain. Data processing: Standard fMRI pre-processing procedures were used. Anatomic regions of interest (ROIs) in MNI space were created for left hippocampus, right hippocampus, left parahippocampal gyrus and right parahippocampal gyrus using WFU PickAtlas software. Averaged fMRI signal time courses were obtained for each ROI and the hemodynamic response function (HRF) for the contrast “novel pictures > familiar pictures” was computed with GLM. Peak stimulus response on the HRF curve was seen between 4.5 and 9 seconds after stimulus onset, these were summed to obtain integrated % signal change. These values were then compared using paired Student t tests.

RESULTS and DISCUSSION: Mean plasma cortisol levels were much higher during hydrocortisone administration (28.9±25.0 µg/dL), compared to placebo condition (8.9±5.9 µg/dL). TRUST MRI data showed that hydrocortisone reduced global venous oxygenation (paired t test, $P=0.01$) (Fig. 1). Fig. 2 shows the group-level fMRI activation maps when comparing “novel pictures” to “familiar pictures” in the placebo trial. Robust activation was detected in the visual cortex, medial temporal lobe and prefrontal cortex. The hydrocortisone trial gave similar activation maps. ROI analysis showed that the fMRI signals in hippocampus were 0.33±0.19% (mean±SD) and 0.23±0.11% for placebo and hydrocortisone trials (Fig. 3), respectively, suggesting a trend of decline but the result from statistical test was still ambiguous ($P=0.137$). Next, we normalized the fMRI signals using global venous oxygenation. Previous studies have shown that resting venous oxygenation is an important modulator of fMRI signal, and they are inversely correlated (1). That is, reduction of global venous oxygenation would result in an over-estimation of fMRI amplitude in hippocortisone trial, which might have partially offset the true neural activity differences. We therefore studied the relationship between fMRI signal and resting venous oxygenation in this study, and confirmed a negative correlation (mixed-effect model analysis, $P=0.04$, one tail). We then normalized the fMRI signals so that the amplitude comparison is conducted under equivalent physiologic conditions. The normalized fMRI signals, calculated as $S_{normalized}(Y_{v}^t-4/\sigma)^{slope}$ (5), were 0.34±0.18% and 0.16±0.15% for placebo and hydrocortisone (Fig 3), respectively, with a definitive difference between the two trials ($P=0.007$). Benefit of normalization in detecting fMRI signal differences was also observed in the parahippocampal ROI (Fig. 3), where the placebo/hydrocortisone difference showed a P value of 0.257 before normalization and it was 0.029 after normalization. Memory assessment conducted after each imaging session showed that the performance in the hydrocortisone trial was slightly poorer than placebo, but did not reach significance ($P=0.1$). In summary, this work revealed that high levels of stress and corticosteroid are associated with a global reduction in venous oxygenation. This observation is in agreement with an earlier report that hydrocortisone reduces baseline BOLD signal (6), but provides a more quantitative measure of brain physiology and demonstrates a spatially non-specific vasocostricitive effect of hydrocortisone. Regional effect of hydrocortisone was also characterized using normalized fMRI, which suggests that hypoxia of hippocampal formation seems to be the underlying mechanism for the declined memory function reported previously (3). The approaches developed in the present study can also be used in other fMRI studies aiming to investigate drug effects on neural activity. Many drugs are vasoactive thus it is critical that the fMRI signal is normalized to account for different physiologic baselines, so that regional effect on neural activity can be separated from global effect on brain vasculature. TRUST MRI may be a useful addition to the fMRI protocol for such purposes. It is non-invasive and of short duration, and does not require challenges such as the inhalation of CO2.