INTRODUCTION
We have developed a system for performing time-series Diffuse Optical Tomographic (DOT) imaging in freely moving rats. In short, this system promises to make possible many of the measurements currently made with functional MRI, PET and similar methods, without the need to anesthetize or otherwise immobilize the animal subject and at much lower cost. The system has good (~1 mm) spatial resolution, high temporal (~17 Hz) resolution and can record continuously for long intervals (tens of minutes, to hours).

Even at this initial stage of development we have combined DOT methods with EEG and behavioral recordings, allowing us to classify optically detected hemodynamic signals according to the electrical state of the rat’s brain and thereby provide a means of validating DOT signals obtained from mobile animals. In a separate poster presented in this conference (190 W-PM), we describe the aspects of instrumentation and measurement for the developed system. In this report, we emphasize methods for DOT image formation and dynamic feature extraction, and present preliminary results of functional imaging in a freely moving rat using an attached head-stage shown in Figure 1.

METHODS
Hypothesis
Our fundamental hypothesis is that the changes in EEG waveforms between theta and LIA (Large Irregular Activity) reflect differences in intrinsic computational style and are accompanied by differences in oxygen demand. We predict, therefore, that DOT measurements of hemodynamic variables averaged over the brain will characteristically differ depending on the state of the hippocampal EEG. We further expect that such differences will be stationary across episodes of theta and LIA, regardless of time in a recording session. Moreover, reflecting the slow (~1-2 Hz) course of hemodynamic change, we expect differences to be magnified if the initial time during each identified theta or LIA episode longer than, say, 2 sec is excluded from analysis, thereby allowing averaging only when the hemodynamic variables have reached a steady state. Finally, we expect that a preliminary analysis of predicted hemodynamic changes will localize such changes to the hippocampus. Our hypothesis is based on the clearer differences between theta and LIA, on the large area of the hippocampus (>15% of rat cortex) and on the finding that the EEG state switches synchronously over the whole hippocampal area (Buzsaki, 2002).

Image Formation and Analysis Environment: NAVI
As presented in Poster no. 685 T-AM, we have developed a program solving environment that allows for the discovery, discovery and analysis of reliable phenomena associated with time-series DOT imaging. NAVI (Near-infrared Analysis, Visualization, and Imaging) is a rich combination of navigation, visualization, and functional NIR data. Using the Linux/Windows computing environments, NAVI offers point-and-click navigation and visualization of data within a flexible file management system that employs wizards to facilitate group data loading, batch processing, automated file system creation, and recording of parameter settings used in data processing. Figure 2 shows one of several display screens that provide for inspection of the temporal dependence of the image pixel data.

RESULTS
We find that time-varying DOT signals averaged over the whole head differ greatly according to the state of the hippocampal EEG. Figure 4 shows that during the 5-12 Hz theta rhythm characteristic of the hippocampal EEG, spatially averaged HbO2Sat values are significantly greater than during the predominant LIA state. Moreover, when the required dwell time in each state is increased, the magnitude of HbO2Sat difference increases. This result is compatible with the presence of a time lag in the hemoglobin response. We have explored by computing the magnitude of EEG-conditioned hemodynamic responses as an increasing fraction of the beginning of each EEG episode is excluded from the computed mean value.

On the idea that it takes for hemodynamic responses to stabilize, we expect differences computed from the residual fraction to be greater, a prediction borne out by curves in Figure 5. The overall response pattern, furthermore, is consistent with MR BOLD findings.

The spatial dependence of the EEG gated hemodynamic response is shown in Figure 6. Arrays A-D are for individual 16 min recording sessions such that A and B are for one rate and C and D for a second rate. Arrays E and F are averages across all 4 of the sessions for different time intervals. Within each array, the map columns are arranged so that the most rostral section is on the left (“head”) and the most caudal on the right (“tail”). The predominant green color over all maps indicates that differences in hemodynamic variables were close to zero over most of the brain, an indirect indication that changes shown in red (increases) or blue (decreases) are not simply explained by changes in systemic blood flow regulation.

Inclusion of the slices reveals several regularities that are sensitive given the origins of the data and that are therefore very encouraging with regard to the imaging method. First, overall pattern of differences for each variable is similar for both rats and quite reproducible across both sessions. Second, major differences are confined mainly to slices 3 and 4 (slice 1 is at the left). Third, these response overall resemble what is expected of an fMRI-derived BOLD response (HBco, HbTot and HbO2Sat go up in theta; HBDeoxy goes down). Finally, the reproducibility of the response is near and symmetric to the midline and quite dorsal, as expected from hippocampal involvement.

CONCLUSIONS
Simultaneous DOT and EEG recordings allow us to see that several well-accepted hemodynamic signals derived from absorption of infra-red light by oxy and deoxy hemoglobin co-vary strongly with the state of the hippocampal EEG. The spatial dependence of the EEG-gated hemodynamic response measured by DOT imaging indicates that the location of these hemodynamic changes are characteristic of the EEG state and that differences in hemodynamic variables are close to zero over most of the brain, an indirect indication that changes shown in red (increases) or blue (decreases) are not simply explained by changes in systemic blood flow regulation.

REFERENCES