Functional Imaging in Freely Moving Rats

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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 therefore 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 sec) time 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 hemodynamics have reached a steady state. Finally, we expect that a preliminary analysis of predicted hemodynamic changes will localize such changes to the hippocampus. Our optimism is based on the clear 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 problem solving environment that allows for the description, discovery and analysis of relevant phenomenology associated with time-series DOT imaging. NAVI, (**N**ear-infrared **A**nalysis, **V**isualization and **I**maging), is a rich constellation of instrument performance monitoring, filtering, image formation, feature extraction, visualization, statistical analysis, and file and database management tools for the examination of functional NIRS 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.

EEG-Gated Image Analysis

To feasibly and efficiently perform EEG-gated image analysis, depicted in Panel A of Figure 3, we have introduced additional graphical interfaces into the NAVI software package (Panel B). This GUI allows display of the EEG-gated Hb responses in the time and spatial domains as a function of the details of the animal's behavioral state/task, selected time period and response duration.

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 HbO_2Sat 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, a notion 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 time 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 indicidual 16 min recording sessions such that A and B are for one rate and C and D for a second rat. 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 larger changes shown in red (increase) or blue (decrease) are not simply explained by changes in systemic blood flow regulation.

Inspection of the slices reveals several regularities that are sensible 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 confine 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 (HbOxy, HbTot and HbSat go up in theta; HbDeoxy goes down). Finally, the preponderance 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 covary strongly with the state of the hippocampal EEG. Early tests of this combined measurement system suggest that the strength of this covariation is very great. Thus, differences between space-averaged hemodynamic signals classified according to EEG state are evident by inspection of colored figures; these impressions are fully corroborated by statistical testing. In addition, we see positional variations in hemodynamic signals as a function of EEG state. The ability to look at a fundamental aspect of function (activity-dependent blood supply regulation) everywhere in the brain at the same time encourages us to believe that we may be able to find regional differences according to the progress of learning of different food-motivated tasks that are known to engage different brain structures.

REFERENCES

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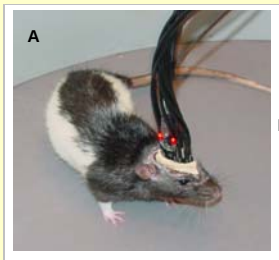

Figure 1. A. Rat with attached DOT/EEG measuring implant. Note the two red LEDs used to track the rat's location. The implant allows for 16 optical source-detection locations with dual-wavelength illumination, plus 12 EEG electrodes, 6 in the dorsal hippocampus on each side of the brain. B. 2-Piece head stage.

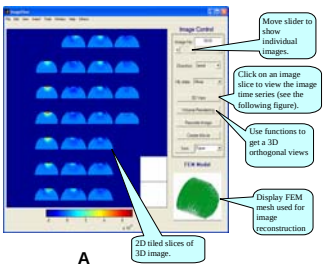

Figure 2. (A) Selected 2-D tiling format for inspection of 3D image data. (b) Pixel time courses of hemoglobin states for the 11th slice illustrated in A.

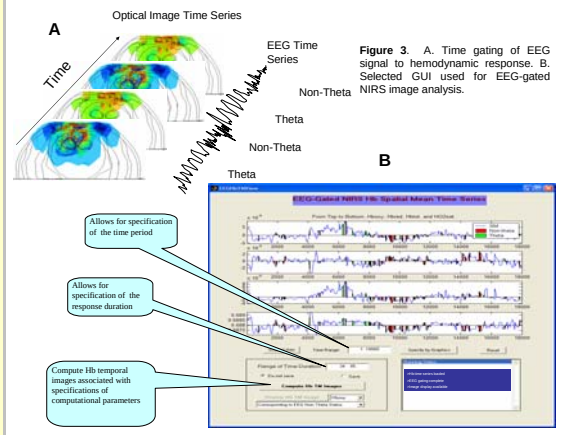

Figure 3. A. Time gating of EEG signal to hemodynamic response. B. Selected GUI used for EEG-gated NIRS image analysis.

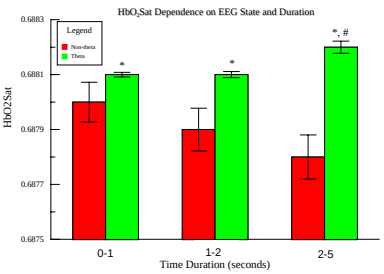

Figure 4. The difference between LIA- and theta-associated HbO_2 signal increases as the duration of the EEG episode increases. * p < 0.01 theta vs LIA. # p < 0.001 1-2 vs 2-5s theta episodes.

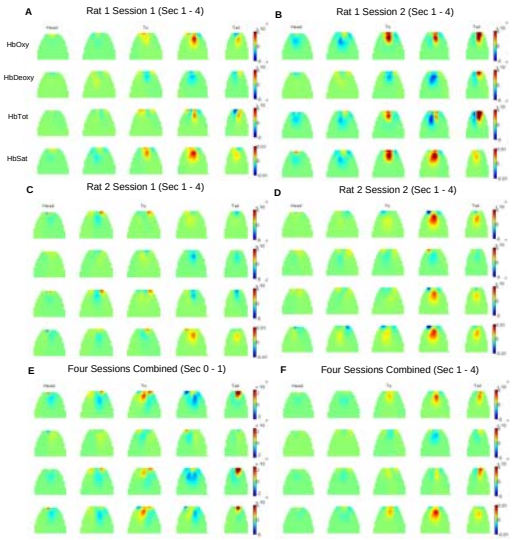

Figure 6. Reconstructed coronal maps of gated-difference Hb response (theta minus LIA). Hb values represent variations in M conc; HbSat values represent changes ± 1%.

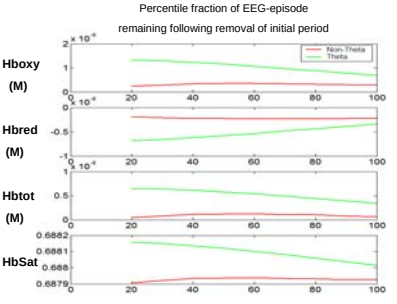

Figure 5. Time and space averaged value of indicated Hb component associated with EEG-conditioned responses lasting between 1-2 seconds. Values shown derived from mean of 23 EEG-episodes.