Functional Multi-Mode Imaging in Freely Moving Rats

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Background & Introduction

Current tomographic imaging methods (e.g., MRI, MEG, PET, SPECT, CT):

- Require subject to be immobile → restricts the ability to explore complex behaviors
- Are expensive, time-consuming, and/or uncomfortable to use

We have developed a Diffuse Optical Tomography (DOT) imager integrated with multi-lead EEG and video tracking that:

- Has good (~1 mm) spatial resolution, high temporal (~17 Hz) resolution, and can record continuously for long intervals (tens of minutes, to hours)
- Is able to make concurrent measurements in freely moving tethered animals, without the need to anesthetize or otherwise immobilize the animal subject
- Has a much lower cost

This set of tools, combined with appropriate behavioral studies, allows us to:

- Classify optically detected hemodynamic signals according to the electrical state of the rat's brain
- Validate DOT signals obtained from mobile animals
- Measure *in vivo* brain dynamics in real time throughout behavioral studies, giving a unique glimpse into the inner workings of the brain at all levels of thought formation and action.

Methods

Instrumentation

Fig. 1 shows a recent version of the two-wavelength DOT instrument (allowing for separation of oxyhemoglobin from deoxyhemoglobin), with up to 9 sources illuminated in a 17-Hz cycle (4 sources used here) and 16 detectors. The 16 detectors (4 of which are co-located with the sources) operate in parallel, so that the reflected light intensity is measured at an aggregate rate of 68 Hz. Each source+detector is connected to the rat's skull via a tether of 16 fiber optic bundles. Included in the tether are wires for EEG recordings and for powering the light emitting diodes (LEDs) used to track the rat's location. In practice we find that the tendency of rats to wind the cabling by turning in a preferred direction is weak, so that sessions > 20 min are possible before mechanical hindrance becomes significant. We have designed an electric commutator rotated under control of the relative position of two differently colored tracking LEDs, and plan to extend this capacity to the optical connections.

Figure 2 shows the two-piece detachable head stage that consists of sixteen 1.5-mm dia. optical fibers, 12-lead EEG electrodes (implanted in the hippocampus) and LED tracking lights. The female part is surgically bonded to the skull, and after healing the male part is attached just prior to each recording session. Optical measurements are made at 760 and 830 nm.

Figure 3 shows the rat implanted with the DOT/EEG head stage. Two tracking LEDs are visible on the right half of the main assembly. The other ends of the black fiber optic bundles are connected to the DOT instrument. From the lack of struggle or vocalization when the male part is connected, it is our belief that this device presents no more of a problem than the usual single cell implants used previously.

Hypothesis

Our fundamental hypothesis is that the changes in EEG waveforms between theta and Large Irregular Activity (LIA) reflect differences in intrinsic computational style and are accompanied by differences in oxygen demand. Therefore, we predict that

- DOT measurements of hemodynamic variables averaged over the brain will characteristically differ depending on the state of the hippocampal EEG.
- Such differences will be stationary across episodes of theta and LIA, regardless of time in a recording session.
- Due to the slow (~1sec) time course of hemodynamic change, these differences will be magnified if the initial time during each identified theta or LIA episode longer than 2 sec is excluded from analysis, thereby allowing averaging only when the hemodynamics have reached a steady state
- 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.

Measurement Protocol

- Typically, the hungry rat is placed for 8 or 16 min in a 1 meter diameter arena where it forages for 25 mg food pellets dropped at 3–4 min^{-1} from an overhead dispenser.
- **Simultaneously**, continuous measurements of DOT, EEG and movement are being acquired. There is an excellent correlation between the behavioral state of the rat and the hippocampal EEG, such that:
  - The 5–12 Hz sine-like theta rhythm occurs during locomotion
  - LIA (the other major EEG state) occurs during eating, grooming or immobility
  - The two EEG states are easily distinguished using power spectral analysis
- Having identified the intervals when the animal is in the LIA and theta states, we use these time signals to gate the hemodynamic response. Each of the Hb signals differs significantly between the two EEG states:
  - Compared to LIA episodes, theta episodes are associated with increased Hboxy, decreased Hbdeoxy, increased Hbtot and increased HbO_2Sat, suggesting that hippocampal activity is elevated during theta

Image Formation and Analysis Environment: NAVI

We have developed software that allows for the 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 filtering, image formation, feature extraction, visualization, statistical analysis, and file and database management tools for the examination of functional DOT data. Image reconstruction of Hb signals is done with the Normalized Difference Method, using a seven-tissue FEM model of the rat head.

EEG-Gated Image Analysis

To feasibly and efficiently perform EEG-gated image analysis, depicted in Figure 4, we have introduced additional graphical interfaces into the NAVI software package (Panel A). 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. An example result is shown in Panel A.

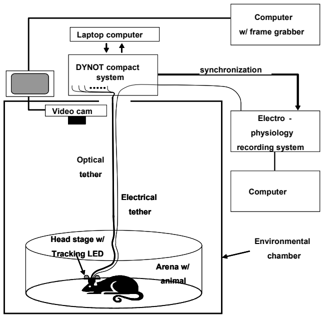

Figure 1. The recording system captures optical data, hippocampal EEG data, and rat location information. The 3 streams are synchronized with a unique event at t = 0, and with signals derived from the DOT device and the camera. The hungry rat is trained to forage for 25 mg food pellets dropped at 0.33 – 0.5 Hz from an overhead feeder. It moves in an unpredictable way over the whole cylinder floor, stopping at times to eat or rest. The state of the hippocampal EEG is used to "gate" DOT data to determine if the overall hemodynamic state of the brain reliably switches, and if so, whether sources of such switches can be localized.

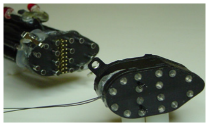

Figure 2. Manufactured and assembled headstage.

Figure 3. A rat implanted with a DOT/EEG headstage.

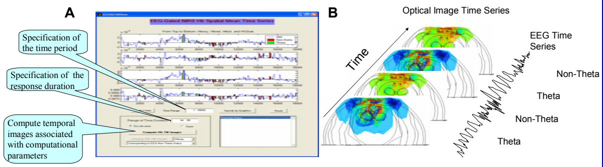

Figure 4. A. Selected GUI used for EEG-gated NIRS image analysis. B. Time gating of EEG signal to hemodynamic response.

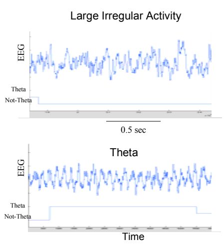

Figure 5. Hippocampal EEG recording for classifying optical recordings.

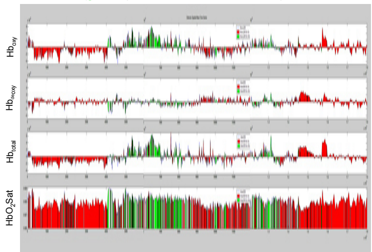

Figure 6. Time series of whole-brain hemoglobin response labeled according to EEG states (green represents theta states while red represents LIA.

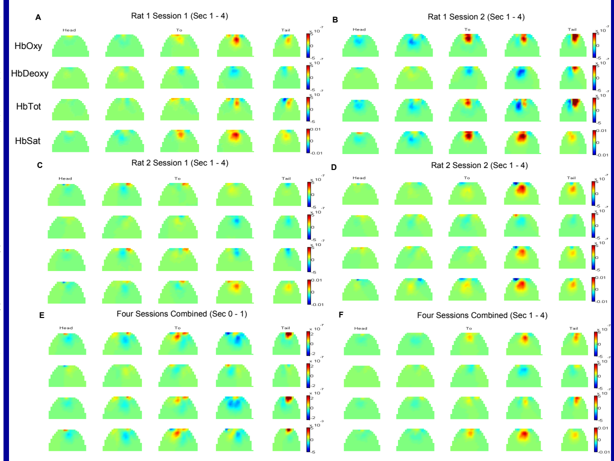

Figure 8. Reconstructed coronal maps of gated-difference Hb response (theta minus LIA). Hb values represent variations in M conc; HbSat values represent changes ± 1%.

Results

Figure 5 shows hippocampal EEG recordings used to classify optical recordings.

- By dividing EEG power in the 6 – 10 Hz band by the power in 1 – 20 Hz band, a theta score is derived.
- Scores above a fixed value indicate theta, scores below a second value indicate LIA (not-theta); scores between the two values indicate an unknown state.

Figure 6 shows time series of whole-brain hemoglobin responses labeled according to EEG states. Color is used to show the theta-score-derived EEG state, such that theta episodes are coded green and LIA in red. By inspection:

- Hboxy tends to be high during theta and low during LIA;
- Hbdeoxy tends to be low during theta and high during LIA.
- Total Hb and Hb saturation are greater during theta.

Taking LIA as the resting state and theta as the reactive state, these changes strongly resemble a BOLD response seen with fMRI, and are in the same direction as seen with fMRI in the human hippocampus during spatial navigation. Therefore:

- The metabolic demands of the brain, and the hippocampus in particular, are greater during theta than in LIA.

We find that time-varying DOT signals averaged over the whole head differ greatly according to the state of the hippocampal EEG. Figure 7 shows that

- During the 5–12 Hz theta rhythm, spatially averaged HbO_2Sat values are significantly greater than during the predominant LIA state.
- When the required dwell time in each state is increased, the magnitude of HbO_2Sat 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.

The spatial dependence of the EEG gated hemodynamic response is shown in Figure 8.

- Arrays A-D are for individual 16-min recording sessions, such that A and B are for one rat 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 seen in 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.
  - 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.
- The general pattern of differences for each variable is similar for both rats, and is quite reproducible across both sessions.
- The major differences are confined mainly to slices 3 and 4 (slice 1 is at the left).
- Overall, these responses resemble what is expected of an fMRI-derived BOLD response (Hboxy, Hbtot and HbO_2Sat go up in theta; Hbdeoxy goes down).
- Finally, the preponderance of the response is close to and symmetric about 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 statistical strength of this covariation is high. 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 different food-motivated tasks that are known to engage different brain structures.

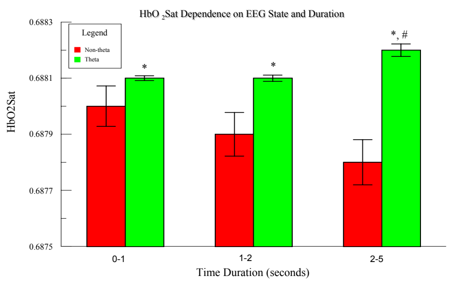

Figure 7. The difference between LIA- and theta-associated HbO_2Sat signal increases as the duration of the EEG episode increases.
* $p < 0.01$ theta vs LIA. # $p < 0.001$ 1-2 vs 2-5s theta episodes.