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) imaging integrated with multi-wavelength EEG and video tracking that:

- Has good (<1 mm) spatial resolution, high temporal (~17 Hz) resolution, and can record continuously for long times (hours to minutes)
- Is able to make concurrent measurements in freely moving behaving 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:

- Classically 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

Introduction

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 collocated with the sources) operate in parallel, so that the reflected light intensity is measured at an aggregate rate of 96 Hz. Each source-detector is connected to the rat’s skull via a 10 fiber optic bundles. Included in the latter are fibers 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 wander by turning towards a preferred direction is weak, so that sessions > 20 min are possible before mechanical tetrode becomes significant. We have designed an electronic commutator mounted 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 headstage that consists of sixteen 1.5-mm dia. optical fibers. 12-led LED electrodes (implanted in the hippocampus) and LED tracking lights. The female port is surgically bonded to the skull, and after heating the male port is soldered to the rat's skull prior to each recording session. Optical measurements are made at 760 and 830 nm.

Figure 3 shows the rat implanted with the DOT/EEG headstage. Two tracking LEDs are visible on the right side of the main assembly. The other ends of the black fiber optic bundles are connected to the DOT instrument. From the lack of movement at the neck and vocalization when the male part is connected, it is our belief that the device prevents no more of a problem than the usual small cell implants used previously.

Hypothesis

Our fundamental hypothesis is that changes in EEG waveforms between theta and large irregular activity (LIA) reflect differences in intracranial computational state and are accompanied by changes in oxygen demand. Therefore, we predict that:

- DOT measurements of hemodynamic variables averaged over the whole brain will be characteristically different depending on the state of the hippocampal EEG.
- Differences will be stationary across episodes of theta and LIA, regardless of time in a recording session.
- Due to the slow (1-sec) time course of hemodynamic change, these differences will be magnified if the initial time during each identified theta or LIA episode is 2 sec or shorter compared with the last 2 sec or is excluded from analysis, thereby allowing averaging only when the hemodynamic changes have reached a steady state.
- A preliminary analysis of predicted hemodynamic changes will locate such changes to the hippocampus.

Our approach 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 0.3–4 min–1 from an overhead dispenser.
- Simultaneously, continuous measurements of DOT, EEG and movement are being acquired.

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 1–2 Hz band, a theta score is derived.
- Scores above a fixed value indicate theta, scores below a second value indicate LIA (not theta); between the two values indicate an unknown state.

Figure 6 shows time series of whole-brain hemoglobin responses related 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:

- Theta tendency is to be high during theta and low during LIA.
- LIA tendency is 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 MRI, 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 HbO2Sat values are significantly greater than during the predominant LIA state.
- When the required dwell time in each state is increased, the magnitude of HbO2Sat differences increases.

This result is consistent with the presence of a time lag in the hemoglobin response, a notion we have explored by computing the magnitude of EEG-conditioned hemodynamic response, as an increasing fraction of the beginning of each episode is eliminated from the computed mean value.

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

- Arrows A,D are for individual 16-min recording sessions, such that A and D are for one rat and C and F for a second rat.

- Arrows E and F are averages across all 4 of the sessions, for different time intervals.

- Within each array, the column averages are so that the most frontal 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 (increases) or blue (decreases) are not simply explained by changes in systemic blood flow.

- Inspection of the sections reveals several regions that are sensitive to 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 (1 cm above the midline, and quite dorsal, as expected from hippocampal involvement).

- Finally, the predominance of the response is to close to and symmetric about the midline, and quite dorsal, as expected from hippocampal involvement.

Conclusions

Synchronous DOT and EEG recordings allow us to see that several well-accepted hemodynamic signals, derived from absorption of infrared light by oxy and deoxy-hemoglobin, convey strongly with the state of the hippocampal EEG.

- Early tests of this combined measurement system suggest that the statistical significance of this correlation is high. Thus:

- Differences between space-averaged hemodynamic signals classified according to EEG state are evident by inspection of colored figures; these impressions are further 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.