Proceedings of the 28th IEEE
EMBS Annual International Conference
New York City, USA, Aug 30-Sept 3, 2006

4D Functional Imaging in the Freely Moving Rat

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Abstract—**We describe a two-frequency diffuse optical tomographic (DOT) imaging and EEG recording system suitable for the study of real-time hemodynamic and neural activities in freely moving rats. The system uses a bundle of 16 optical fibers that both deliver light and capture its reemission. This bundle runs in parallel with a cable carrying EEG signals from 16 microelectrodes. Both data collection arrays terminate in a precision-machined cap that is surgically attached to the skull. Free movement is enabled by suspending the cables with an elastic cord. Rats are also tracked with a video system so their behavior can be compared to hemodynamic and neural activity. Optical measurements are done with 760 and 830 nm laser diodes using a time-multiplexed, frequency-encoded illumination scheme at a source-switching speed of 68 Hz. EEG, optical and video data are all timestamped with the same clock, ensuring information synchrony. Automated optical system set-up and control is done with a LabVIEW interface that allows on-the-fly adjustment of gain, data integrity checks and system calibration, among other functionalities. EEG recording is done with a Neuralynx (Tucson, AZ) recording system. Collected optical data are converted to volumetric images either in real-time or offline. The integrated system includes comprehensive image formation, display and time-series analysis software suitable for processing data independently or in combination.**

I. INTRODUCTION

A key feature of fMRI is its ability to view the entire brain all at once. Nevertheless, there are critical limitations to fMRI methodology. For one thing, the hardware is very expensive and hard to maintain. It is true that the costs are likely to continue to decrease, but from our present vantage it is difficult to imagine ad lib access by large number of investigators. A second problem is the need to immobilize humans or to correct for head movements [1] or to anesthetize animal subjects in order to make recordings. A third difficulty is intrinsic to the physics of MRI; it is extremely difficult to make electrical measurements such as EEG recordings at the time of MR acquisition and during the subsequent relaxation, at the very time that such recordings are most interesting.

Manuscript received July 7, 2006. This work was supported in part by NIH Grants R41-NS50007, R43-NS49734 and R01-NS20686. M. Holzer, J. Barry and R. Muller are in the Dept of Physiology and Pharmacology; H. Graber, R. A. Abdul, and R. Barbour are in the Dept of Pathology, SUNY Downstate Medical Center, Brooklyn, NY 11203; Y. Pei is with NIRx Medical Technologies, LLC Glen Head, NY 11545.

We therefore would like to begin to apply the alternative of diffuse optical tomography (DOT) imaging to the study of perception, learning and memory. In principle, DOT solves the stated problems with fMRI although it presents certain difficulties of its own. Thus, the hardware and maintenance are nominal compared to fMRI, there is no need to immobilize or anesthetize the subject and the lack of interaction between photons and electrical charge means that DOT measurements can be made simultaneously with EEG, evoked potential or single cell recordings. Using a locally built device of nominal cost, we provide in Results validating evidence for DOT measurements in freely moving rats by using simultaneous EEG recordings to separate DOT signals according to this independent variable. Moreover, we can obtain *enhanced* BOLD responses with our instantiation of DOT; using two wavelengths allows concurrent estimates of both oxy- and deoxy-hemoglobin, in turn permitting estimates of total hemoglobin and hemoglobin saturation, quantities that require additional, sophisticated methods to obtain along with fMRI [2]. It is essential to note also that the 17 Hz framing rate of the DOT instrument we use provides more than enough temporal resolution for BOLD responses.

II. DESIGN AND PROCEDURE

In initial experiments, we detected changes in hemodynamic variables that occur in association with transitions between the two main EEG patterns seen in the rat hippocampus. The tomographic instrument is a recent version of devices constructed in the Barbour laboratory for imaging a variety of tissues [3-5]. This two-wavelength instrument (allowing for separation of oxyhemoglobin from deoxyhemoglobin) has 4 sources and 16 detectors. The sources are illuminated in a 17 Hz cycle. The 16 detectors (4 of which are multiplexed with the sources) operate in parallel so that the reflected light intensity is measured at an aggregate rate of 68 Hz. In use, each detector or 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 (LED's) 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 (see Figure 1 for recording system schematic).

The DOT/EEG head stage is made of 3 parts. The upper, male part receives the 16 fiber bundles whose ends protrude by 3 mm. This upper piece also has electrical connectors for EEG recordings and LED power. To begin recording, the upper part is inserted into the lower part formed by the other

2 parts. Mechanical connectors are used to fix the ensemble in place. To disconnect the rat, the mechanical connections are undone and the upper part removed.

The 2 lower parts are glued together during assembly and later permanently attached to the rat's head during a surgical procedure similar to that used routinely to allow single cell and EEG recording. The rat is anesthetized with 45 mg/kg Nembutal and put in a stereotaxic instrument. Holes for the EEG electrodes and anchoring holes are drilled through the skull. Self-tapping screws are inserted into anchoring holes. The bottom piece is then lowered into place such that a set of 6 EEG electrodes penetrates each brain hemisphere and the contoured lower surface comes into contact with the top of the skull. The bottom piece is secured to the anchoring screws with dental cement. The permanent implant contains polished transparent plastic light tubes to bring the optical connection as close to the skull as possible. All EEG wires are presently aimed at the hippocampus and are cut to different lengths so that at least one pair is very likely to straddle the CA1 cell layer, enhancing the quality of electrical recordings.

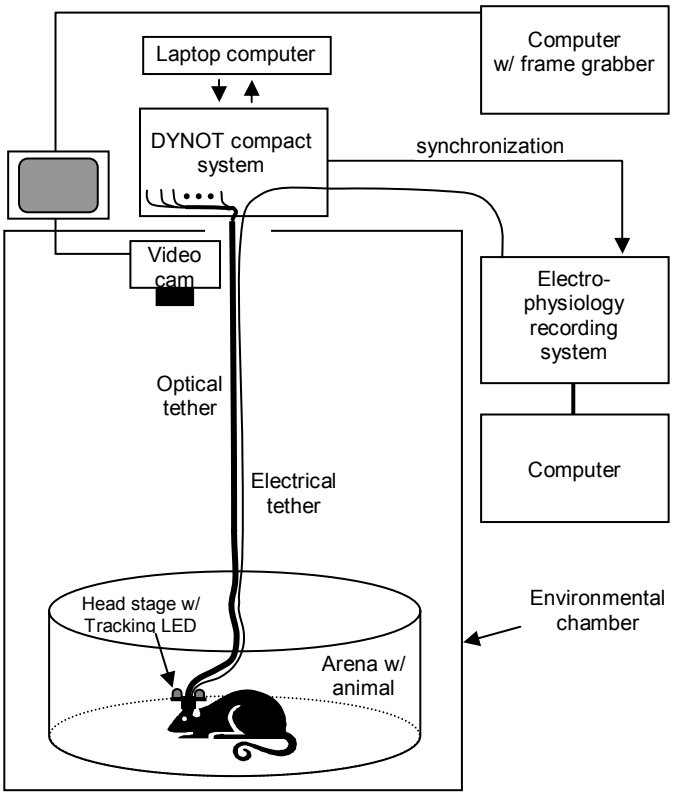

Figure 1. The recording system captures 3 data streams, namely optical information with fiber bundles, hippocampal EEG information with electrical wires and location information with an overhead video camera. The 3 streams are synchronized with a unique event at t = 0 and with signals derived from the 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 well-known relationship between locomotion and the hippocampal theta rhythm on the one hand and between any of quiet alertness, eating, grooming and the large irregular activity (LIA) on the other hand ensures we will find episodes of both of the predominant EEG patterns within long (~20 min) recording sessions. As seen in Figure 2, 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.

The implant components are manufactured with a Roland EGX-300 etching/milling machine controlled by a PC. Design is done with Rhino modeling software and converted to "printing" commands for the EGX-300 with Visual Mill software. The design/manufacturing cycle is easy to implement and rapid.

III. Results

Our preliminary work was done as hungry rats retrieved small food pellets scattered from above into a 0.75 M diameter cylinder at a rate of 2 – 3 per min. The duration of walking, eating and resting episodes is variable. Locomotion and postural shifts are nearly always accompanied by the 5 – 12 Hz "theta" rhythm in hippocampal EEG. In contrast, "housekeeping" activities such as eating, grooming, urination or defecation and quiet alertness are accompanied by "large irregular activity" (LIA) [6]. The waveforms of theta and LIA are distinct so that episodes of each type can be identified using power spectral methods.

Our fundamental hypothesis is that the changes in EEG waveform between theta and LIA reflect differences in 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, for example, 2 sec is excluded from analysis and thereby allowing averaging only when the hemodynamics have reached a steady state. Finally, we expect that a preliminary imaging analysis of hemodynamic changes will localize such changes to the vicinity of 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 [7].

The basic initial finding (Figure 2) suggests that Hb_{oxy} tends to be high during theta and low during LIA whereas Hb_{deoxy} has the opposite relationship to EEG state. In addition, total Hb and Hb saturation are greater during theta. Taking LIA as the baseline and theta as the active state, these changes strongly resemble a BOLD response seen with fMRI and in fact are in the same direction seen with fMRI in

the human hippocampus during spatial navigation but not landmark-based navigation on a virtual four-arm (+) maze [8]. We therefore suggest that the metabolic demands of the brain in general and the hippocampus in particular are greater during theta than LIA. A simple statistical analysis confirms that hemodynamic variables are strongly modulated by theta state; the probability of observing the difference by chance is virtually zero in each case.

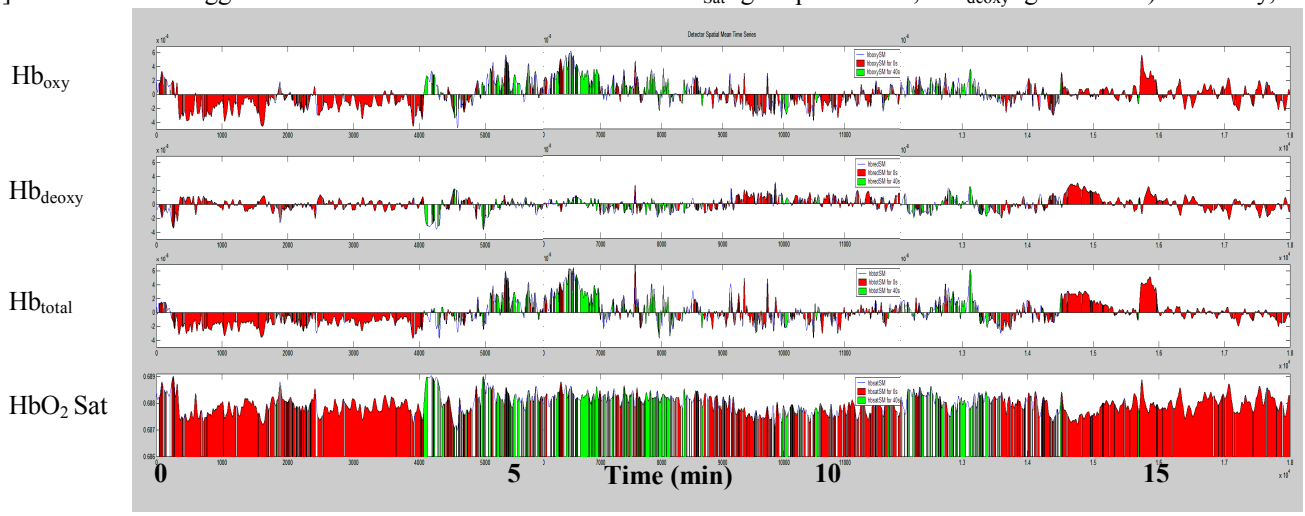

Figure 2. Hb responses during theta (green) and LIA (red) periods. The increase of Hb_{oxy} and Hb_{total} and the decrease of Hb_{deoxy} during theta are evident. Average HbO_2 saturation also goes up during theta. Statistical tests strongly corroborate the clear differences seen by inspection.

Using these data we made tomographic images by applying a first-order solution to the linear perturbation formulation of the inverse diffusion problem, using a finite element mesh model of the rat head [9]. Using established methods, we made tomographic slice images of differences of hemodynamic variables between EEG states with LIA as the reference so, for example, an Hb_{oxy} increase during theta is positive. The difference is color coded and plotted as a function of computed position in the brain. We present coronal sections in Figure 3 but horizontal or sagittal sections as easily produced. Arrays A and B are averages 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"). Also within each array, the map rows from top to bottom are in the order Hb_{oxy}, Hb_{deoxy}, HB_{Tot} and Hb_{Sat}. The dominant green for all maps indicates that differences in hemodynamic variables are close to zero over most of the brain, suggesting that larger changes shown in red (for increases) or blue (for decreases) are not explained simply by changes in systemic blood flow.

In Figure 3B, difference values were obtained by finding the duration of each theta or LIA episode and averaging each hemodynamic quantity only between 1 sec and at most 4 sec into the episode. Not shown is the fact that the overall pattern of differences for each variable is similar between rats and reproducible between sessions. Combining 4 sessions, major differences are confined mainly to slices 3 and 4 (slice 1 is at the left). Third, these response overall resemble an fMRI-derived BOLD response (Hb_{oxy}, Hb_{Tot} and Hb_{Sat} go up in theta; Hb_{deoxy} goes down). Finally, the

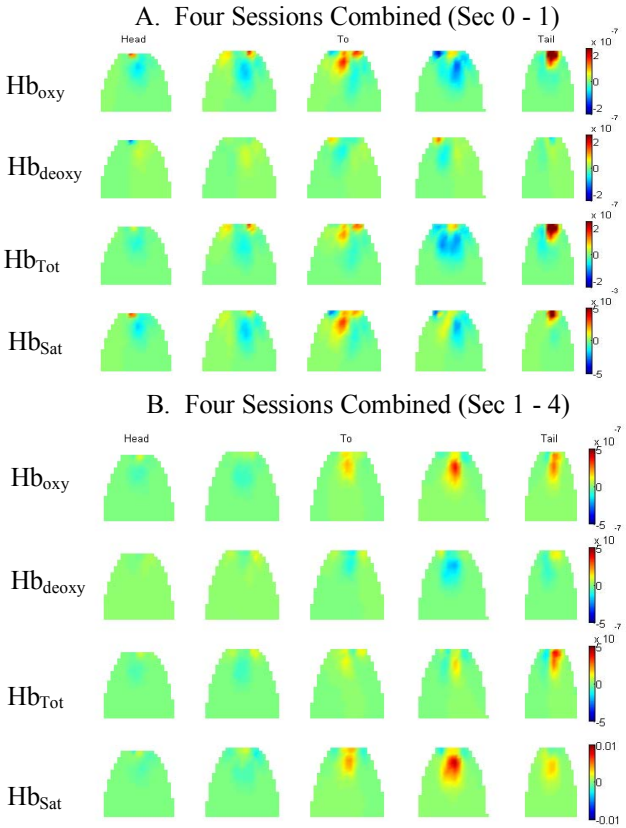

Figure 3. Reconstructed coronal maps of gated-difference Hb responses (theta minus LIA).

preponderance of the response is near and symmetric to the midline and quite dorsal, as expected from hippocampal involvement.

As stated above, given the reasonable reproducibility of individual sessions it is not surprising that the averaging process yields combined maps resemble the original data. In contrast, it is very gratifying that averaging each hemodynamic variable between 0 sec and 1 sec into either LIA or theta episodes reveals an entirely different pattern. In array A, we see an indication that Hb_{oxy}, Hb_{Tot} and Hb_{Sat} go down early in the transition into theta, as expected if the presumed elevated hippocampal processing accompanying theta incurs an oxygen debt that is later satisfied by regulatory mechanisms, resulting in an overshoot compared to the basal LIA level. This interpretation closely follows the most widely accepted accounts of the processes that underlie fMRI BOLD responses and is, in our estimation, a reason for great optimism.

We did not see the early, inverse BOLD response at the beginning of EEG episodes when we analyzed the space-averaged data. A plausible explanation of this failure is visible in array A of Figure 3 where the inverse response in slice 4 is flanked by a direct BOLD-like response in slices 3 and 5. It may be that the space average cannot detect the inverse response because it is not homogeneous. Overall, however, it is our contention that the spatial differences of EEG-gated differences in hemodynamic variables so far conform to expectations. Accordingly, we are encouraged to apply these methods to look for learning-induced changes in different brain regions [8,10,11].

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