EEG-Gated DOT Imaging in Freely Moving Animals

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INTRODUCTION

Many tomographic imaging methods (e.g., MRI, MEG, PET, SPECT, CT) require the subject to be immobile, so that gross movements (e.g., walking) are simply incompatible with data acquisition. This limitation severely restricts the ability to tomographically explore many complex behaviors carried out by humans and animals; only the freely moving condition allows for the full range of behaviors exhibited by a species. We report here and in a second poster presented in the conference (R. L. Barbour *et al*., "Functional Imaging in Freely Moving Rats") the development of an integrated system combining optical-DOT imaging, multi-lead EEG recordings, and video tracking that enables concurrent measurements in freely moving tethered animals.

METHODS

Instrumentation

Shown in Fig. 1 is a recent version of the DOT instrumentation developed by the SUNY team for imaging a variety of tissues. This two-wavelength instrument (allowing for separation of oxyhemoglobin from deoxyhemoglobin) has up to 9 sources (4 sources used here) 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 (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, however, 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 a photograph of 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. After healing, the separable male part is attached just prior to each recording session. Optical measurements are made at 760 and 830 nm using 16 detector positions, 4 of which are also sources; the framing rate is 17 Hz. Image reconstruction of Hb signals is done with the Normalized Difference Method of Pei et al. [1] as detailed by Schmitz et al. [2], with a seven-tissue FEM model of the rat head.

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. Visible to the right of the optic bundles is a gray electrical cable. The dental cement used to attach the implant to the skull is visible as an off-white band. 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.

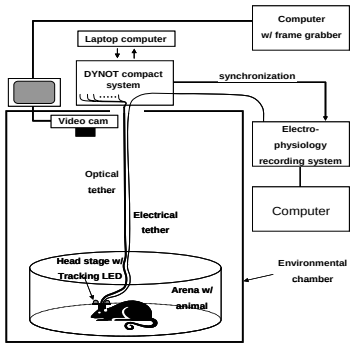

Figure 1. The recording system captures 3 data streams, namely optical information with fiber bundles, hippocampal EEG information with electrical wires and location information with an overhead video camera. The 3 streams are synchronized with a unique event at (t = 0) and with signals derived from 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, or 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 (16 min) recording sessions. 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.

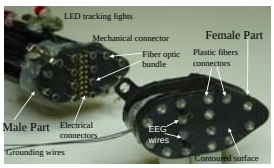

Figure 2. Manufactured and assembled headstage.

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

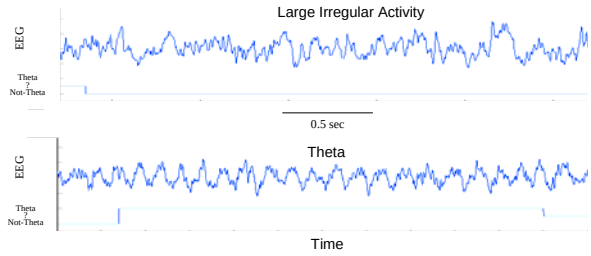

Figure 4. Hippocampal EEG recording for classifying optical recording.

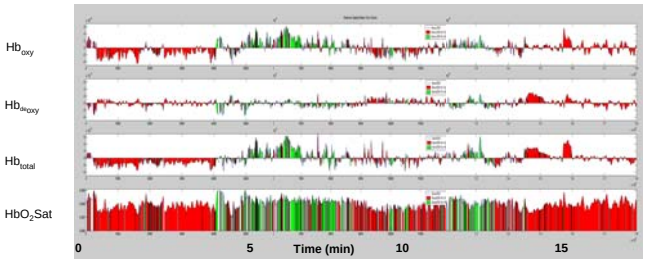

Figure 5 . Time series of whole-brain hemoglobin response labeled according to EEG states, in which green represents theta states while red represents LIA.

Measurement Protocol

In a typical recording session, 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.

During this entire time, time series of DOT, EEG and movement are being acquired. Note that 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, and the other major state (LIA) occurs during eating, grooming or quiet 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 Hb_{oxy}, decreased Hb_{deoxy}, increased Hb_{tot} and increased HbO2Sat suggesting that hippocampal activity is elevated during theta.

RESULTS

Figure 4 shows hippocampal EEG recordings used for classifying optical recording, while Figure 5 shows time series of whole-brain hemoglobin responses labeled according to EEG states.

Table 1 presents the results of t-tests for space-averaged hemodynamic variables gated by the hippocampal EEG. Note that the sign of the difference between the LIA average and theta average values for each parameter is as expected for a BOLD response, indicating an increase in oxygen utilization of the sort frequently obtained from fMRI.

Figures 6 shows the spatial dependence of the difference between HbO2Sat (left) and Hb_{tot} (right) signals gated by EEG state responses during two different recording sessions for the same animal. We think that these prelimary data strongly suggest that the EEG-gating of hemoglobin signals is reproducible across time. Inspection reveals activation of the cerebellum, hippocampus and prefrontal cortex, all structures implicated in control of locomotion.

CONCLUSIONS

Real-time imaging in freely moving animals is technically feasible and provides for Hb responses that are repeatable and spatially distinct when gated to different hippocampus states.

REFERENCES

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This work was supported in part by the National Institutes of Health (NIH), under Grants R41-NS050007 and R43-NS49734.

Table 1. t-tests for space-averaged hemodynamic variables gated by the hippocampal EEG.

Hemoglobin State	EEG classification	Mean (M)	Standard deviation (M)	Number of time frames	t-statistic (df)	p-value
Hb_{oxy}	Non-Theta	-6.18e-9	1.46e-8	7976	-25.27 (935.92)	2.39e-104
	Theta	1.06e-8	1.86e-8	828		
Hb_{deoxy}	Non-Theta	1.93e-9	9.38e-9	7976	16.80 (1056.43)	2.57e-56
	Theta	-3.25e-9	8.34e-9	828		
Hb_{tot}	Non-Theta	-4.25e-9	1.55e-8	7976	-15.98 (929.08)	5.93e-51
	Theta	7.37e-9	2.03e-8	828		
HbO_2 Sat	Non-Theta	0.68787	0.00029	7976	-29.37 (1026.08)	4.29e-138
	Theta	0.68817	0.00028	828		

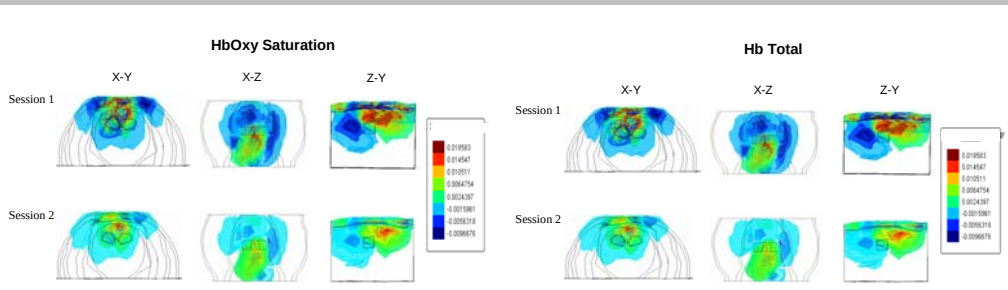

Figure 6. Each stacked 3-D map shows the color coded spatial distribution of the EEG-selected difference in a signal seen from coronal (X-Y), horizontal (X-Z) and sagittal (Z-Y) views. The two key points are the distinct variations of both the example signals and the reproducibility of the signals from session 1 to session 2.