Functional Imaging in Freely Moving Rats

Randall L. Barbour,[1,2] Yaling Pei,[2] Harry L. Graber,[1,2] Rehman Ansari,[2] Christoph H. Schmitz,[1,2] Matthew Holtzer,[3] Jeremy Barry,[3] and Robert Muller[3]

Departments of [1]Pathology and [3]Physiology, SUNY Downstate Medical Center, 450 Clarkson Avenue, Box 25, Brooklyn, NY 11203 US
[2]NIRx Medical Technologies, LLC., 15 Cherry Lane, Glen Head, NY 11545 US
randall.barbour@downstate.edu

Abstract: We have developed an integrated DOT-EEG and behavioral recording system that provides, through a tethered head stage, concurrent recording in freely moving rats. Results show that EEG-gated signals (theta, LIA) have spatially distinct hemodynamic responses.

OCIS codes: (060.2350) Fiber optics imaging; (120.3890) Medical optics instrumentation; (330.5380) Physiology.

Introduction: Common to many tomographic imaging technologies used in clinical and investigative studies (e.g., MRI, MEG, PET, SPECT, CT) is the need to render the subject immobile. Gross movements (e.g., walking) are simply incompatible with data acquisition. This limitation, however, can severely restrict the ability to explore a range of complex behaviors common to our everyday experience. In fact, it is only the freely moving state that embodies the full range of behaviors exhibited by a species. In this study we have developed an integrated optical-DOT imaging, multi-lead EEG, and video tracking capability that allows for concurrent measurements in freely moving tethered animals.

Methods: Figure 1 shows a photograph of the two-piece detachable head stage we have developed, consisting of 16 1.5-mm-dia. fibers (source and detector), 2 6-lead EEG electrodes (implanted into the left and right hippocampus), and LED motion tracking lights. The female part is surgically bonded to the cranium and, after allowing for wound healing, the male part is attached just prior to initiating imaging studies in freely moving animals. Figure 2 shows the completed unit attached to a mobile animal.

Figure 1. Optical-EEG Head Stage

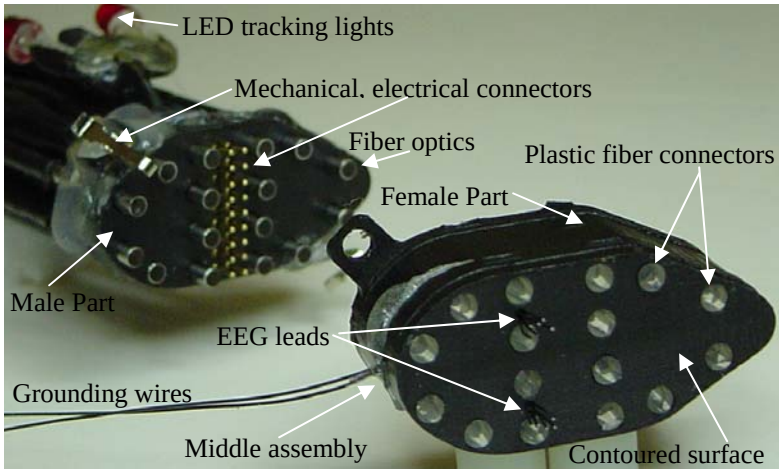

Figure 2. Photograph of rat with attached head stage.

Measurement Protocol: A typical recording session involved placing the animal in a 1-meter-diameter arena and allowing the animal to forage for food pellets introduced from an overhead dispensor, all the while time-series DOT (17 Hz framing rate), EEG, and video recording were occuring. While immobile, eating or grooming, the hippocampus exhibits large irregular amplitude (LIA) EEG signals and theta

rhythms during locomotion. Using power spectral analysis methods, these two rhythms can be easily distinguished. Having identified the time intervals when the animal is in LIA and theta, we use these signals to gate the hemodynamic response. Optical measurements were performed at 760 and 830 nm using 16 detectors and 4 source positions with a 17 Hz framing rate. Image reconstruction of Hb signals was accomplished using the Normalized Difference Method described by Pei et al. [1], as detailed by Schmitz et al. [2], with a seven-tissue FEM model of the rat head.

Results: Figure 3 shows the time-dependence of the spatially averaged image series result for the different forms of Hb color coded to correspond to periods of LIA (red) and theta (green).

Figure 3. Spatial mean Hb time series. Green (theta), red (LIA)

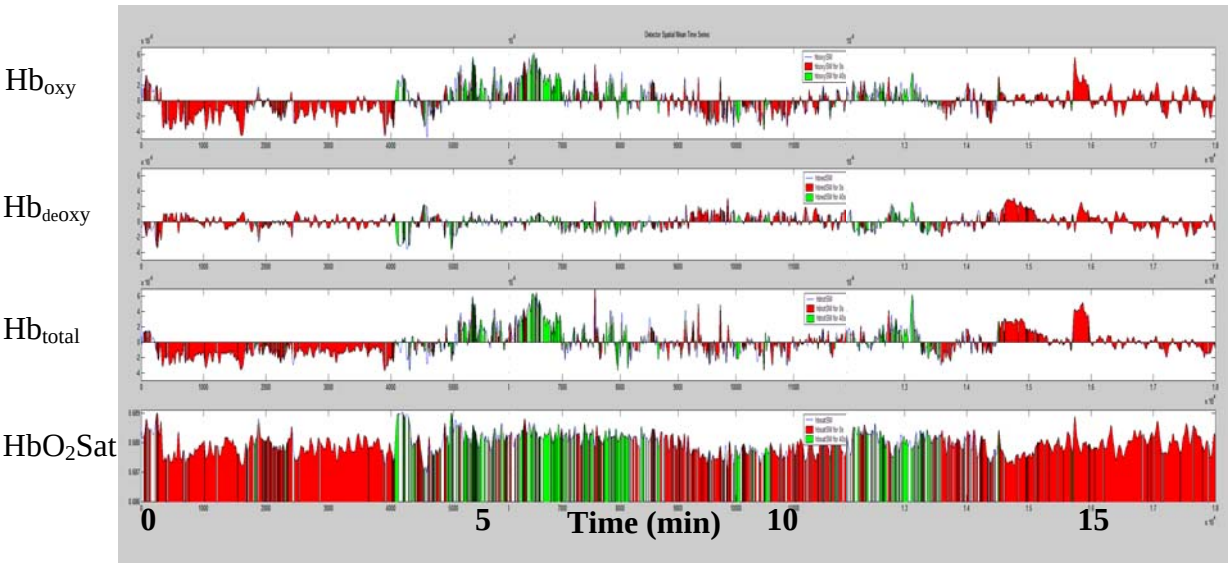

Table 1 shows the results of mean significance tests for gated signals averaged for time periods > 2 sec.

Table 1. Significance test on time, space averaged gated Hb response.

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		

Inspection reveals that the different EEG states produce responses that are significantly different for all forms of Hb. Specifically, we find that compared to the LIA period, theta rhythms are associated with an increase in oxyHb, decrease in deoxyHb, increase in totalHb and HbO_2Sat. These finding suggest that during locomotion, a hightened state of neural activity is occuring.

Figures 4 and 5 show the spatial dependence of the gated Hb responses (difference image) acquired from two different imaging sessions on the same animal.

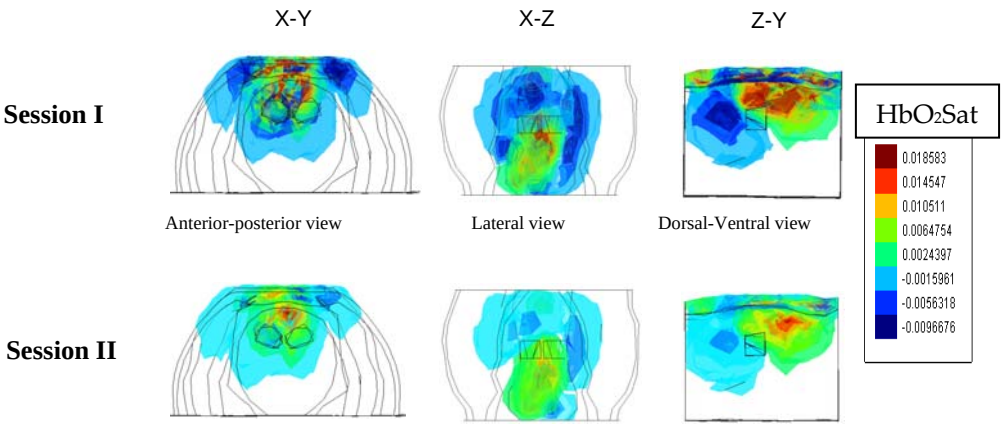

Figure 4. Orthogonal views of HbO_2Sat difference (theta minus LIA) image.

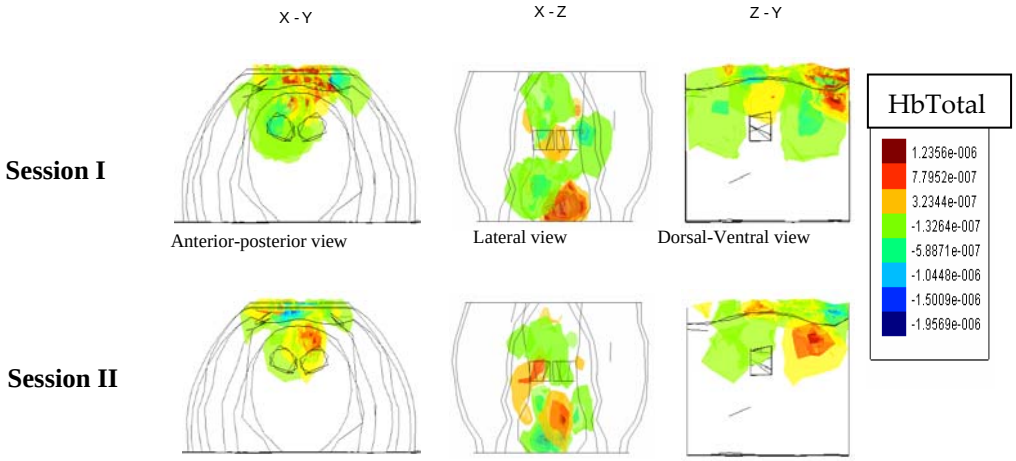

Figure 5. Orthogonal views of Hb_{total} difference (theta minus LIA) image.

Inspection shows that the spatial response profiles are repeatable and indicate activation of the cerebellum, hippocampus and prefrontal cortex, which are the expected structures involved in locomotion.

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

References:

[1] Y. Pei, H. L. Graber, and R. L. Barbour, "Influence of systematic errors in reference states on image quality and on stability of derived information for DC optical imaging," *Applied Optics* **40**, 5755-5769 (2001).

[2] C. H. Schmitz, D. P. Klemer, R. E. Hardin, M. S. Katz, Y. Pei, H. L. Graber, M. B. Levin, R. D. Levina, N. A. Franco, W. B. Solomon, and R. L. Barbour, "Design and implementation of dynamic near-infrared optical tomographic imaging instrumentation for simultaneous dual-breast measurements," *Applied Optics* **44**, 2140-2153 (2005).