Simultaneous functional diffuse optical tomography and EEG in freely moving rats

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Introduction

Functional imaging technologies allow real-time visualization of brain activity that enhances the study of learning, memory and disease. Current technologies, such as fMRI, have drawbacks including: 1) cost of use in time and money; 2) physical constraints that prevent the experimental subject from fully interacting with the environment; and 3) an inability to take simultaneous supplementary measurements, such as EEG.

Diffuse Optical Tomography (DOT) is a novel non-invasive functional imaging technology designed to visualize real-time relative changes in oxygenated and deoxygenated hemoglobin (OxyHb and DeoxyHb, respectively) levels in the brain over extended times. Unlike fMRI, which tracks changes in DeoxyHb levels in the brains of immobilized subjects, DOT is less costly, allows the experimental subject to freely interact with the environment and can be combined with EEG and behavioral methods to investigate changes that take place in the brain when rats learn and perform different tasks.

Methods - Rat Foraging

To demonstrate the capabilities and fidelity of DOT + EEG recordings, a DOT imager was added to an experimental setup (Fig 1) used for place cell recordings. The imager was constructed with two lasers, 9 sources illuminated at 7Hz, and 16 detectors that detect light following source illumination. A tether consisting of fiberoptic bundles carried the optical signal from the lasers to the rat skull and from the skull to detectors via a headstage that also contained EEG wires (Fig 2A). A plastic 'Slinky" was used to suspend the tether removing its weight from the rat.

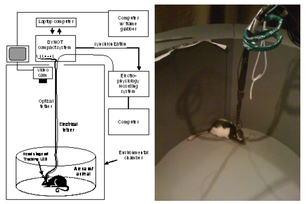

Figure 1: Experimental setup for rat foraging experiments. Schematic (left) and actual recording session (right) are shown.

Foraging experiments involved training hungry rats to find food pellets scattered onto a 0.75M diameter cylinder at a rate of 2-3 per minute (Fig 1). Following training, a DOT/EEG implant (Fig 2B) was affixed to the surface of the rat skull under Nembutal anesthesia and the rat was allowed 1 week to recover. A typical experiment involved attaching the male part of the headstage to the implant (Fig 2A), placing the rat inside the cylinder and allowing it to forage for 15-20 minutes while DOT and EEG data were recorded (Fig 2C).

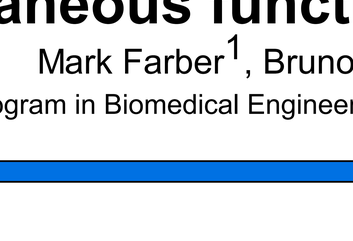

Figure 2: Implant design. A) Implant affixed to the surface of the rat skull interfaces with fiberoptic bundle and EEG tether. B) Updated interface improves contact stability between implant and fiberoptic bundle. C) Foraging rat connected to fiberoptic bundle.

Methods - 'Phantom' Experiments

DOT data fidelity was significantly improved by replacing spring-based connectors (Fig 2A) with a nut/bolt configuration (Fig 2B). Custom-built "phantoms", each consisting of an implant affixed to silica bricks with known optical properties (Fig 3A) was attached to the DOT imager and manipulated to mimic rat foraging. Images were reconstructed using an FEM model (Fig 3B), averaged across the entire volume, and plotted (Fig 3C). The new nut/bolt configuration was not affected by rat-like movements of the phantom.

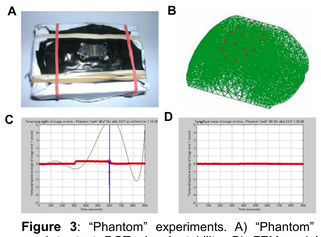

Figure 3: "Phantom" experiments. A) "Phantom" used to test DOT signal stability. B) FEM model used to generate tomographic images from DOT data. C) Image spatial mean time series result from dynamic "phantom" experiments with spring-based interface. D) Same result for updated nut/ bolt interface.

Methods - Foraging Data Analysis

EEG transitions into LIA (Fig 4, top) and theta (Fig 4, bottom) were detected using Matlab code. All LIA and theta epochs >4sec were identified and synchronized with DOT signals based on timestamps recorded during data acquisition. DOT images extracted from >4sec LIA epochs were subtracted from the closest following >4sec theta epochs images, and averaged across all epoch pairs in a session. The resultant image time series was temporally averaged for the intervals 0-1 seconds and 1-4 seconds, yielding two sets of images for each session.

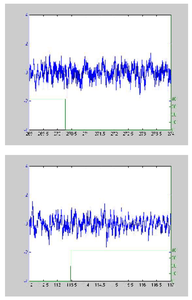

Figure 4: Identification of EEG transitions to LIA and theta. Representative EEG time series (blue) and ouput of program (green) designed to detect transitions to LIA (top) and theta (bottom).

Results - Rat Foraging Experiments

Resulting theta-LIA difference images, temporally averaged across 0-1 seconds and 1-4 seconds following EEG state transitions, are plotted for example individual sessions in Figure 5A. The corresponding spatially-averaged time series preceding and following a transition into one epoch of theta or LIA within the individual sessions are plotted in Figure 5B (blue) with the calculated EEG state (green). Results averaged across all epochs and sessions in each animal are shown in Figure 6.

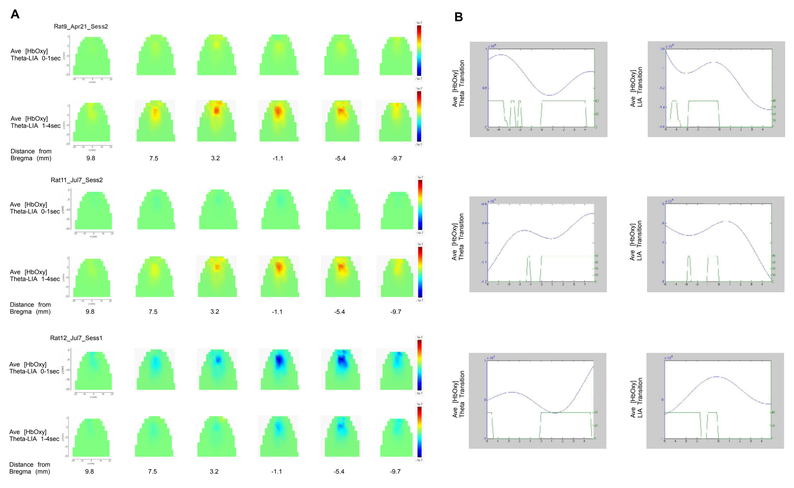

Figure 5: Temporal and spatial averages highlight differences in the brain's hemodynamic state when EEG is in theta vs. LIA. A) Coronal brain slice images reconstructed for theta-LIA differences of HbOxy over the intervals from 0-1sec and 1-4 sec after transition pairs. The top two rows of images are for rat 9, the middle for rat11 and the bottom for rat 12. A BOLD-type response, characterized by an initial decrease in [HbOxy] in the 0-1sec interval after entering the EEG states followed by a strong increase during 1-4sec increase, was observed in nearly all sessions (20/ 23). B) Representative time series of spatial means (blue) during transition into theta (left) and LIA (right) epochs on the same time scale as the theta score (green). Upon transition into theta, the [HbOxy] spatial mean time series resembles a BOLD-type response observed with fMRI, whereas transitions into LIA in the EEG led to a consistent decrease in the spatial mean time series.

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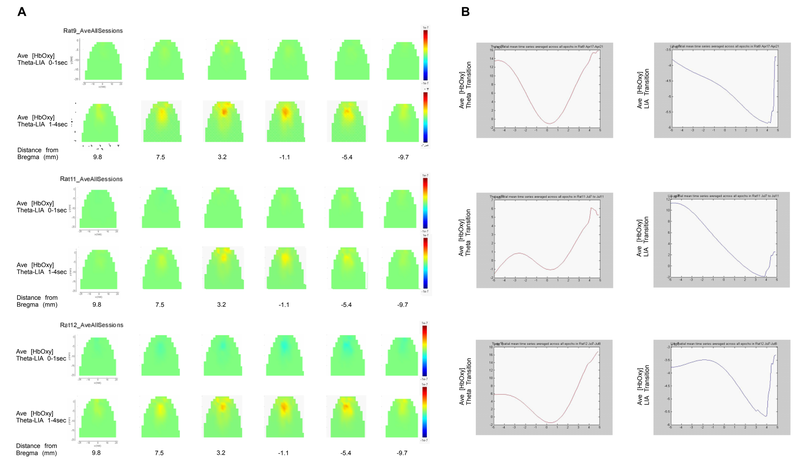

Figure 6: Temporal and spatial averages highlight differences in the brain's hemodynamic state when EEG is in theta vs. LIA. A) Coronal brain slice images reconstructed for theta-LIA differences of HbOxy over the intervals from 0-1sec and 1-4 sec after transition pairs, and averaged across all sessions. The top two rows of images represent the average across all sessions for rat 9, the middle for rat11, and the bottom for rat 12. A BOLD-type response, characterized by an initial decrease in [HbOxy] in the 0-1sec interval after entering the EEG states followed by a strong increase during 1-4sec increase was observed in nearly all sessions (20/ 23). B) Time series of spatial means during transition into theta (left) and LIA (right) epochs averaged across all sessions. Upon transition into theta, the [HbOxy] spatial mean time series resembles a BOLD-type response observed with fMRI, whereas transitions into LIA in the EEG led to a consistent decrease in the spatial mean time series.

Methods - Acute Procaine Injection Experiments

Rats anesthetized with 20% urethane (1.2mg/kg) underwent surgery for implantation of an updated implant with a cannula. DOT images were recorded for 1 hour (baseline), followed by injection of 1uL of 20% procaine into the hippocampus (-3.8mm behind bregma; 3.0mm left of midline). Images were reconstructed and temporally averaged across 5-6min before injection, 5-6min after injection, and 60-61min after injection.

Results - Acute Procaine Injection

Baseline DOT recordings demonstrated that urethane causes the HbOxy levels to cycle from slightly below to slightly above baseline. Injection of procaine caused a localized change in HbOxy distribution in the brain, such that there was less OxyHb present in the area of injection when compared to other surrounding areas (Fig 7).

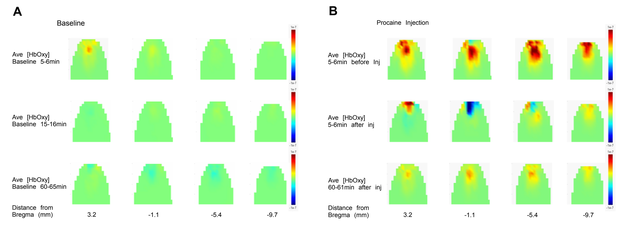

Figure 7: Procaine injection induces local hemodynamic changes. A) Baseline temporal mean images averaged across 5-6min (top), 15-16min (middle), and 60-65min (bottom) after start of recording. Baseline HbOxy measurements in the entire brain fluctuated uniformly about 0. B) Temporal mean images preceding and following injection of 1uL of 20% procaine solution: 5-6min before injection (top); 5-6min after injection (middle); 60-61min after injection (bottom). An asymmetrical decrease in HbOxy near the site of injection (-3.8mm) was observed 5-6min after procaine injection, but was not detected 60min after injection.

Summary

Results demonstrate that DOT imaging combined with EEG is able to reliably distinguish between two distinct metabolic states that depend on the hippocampal EEG activity in a freely moving rat. Furthermore, preliminary DOT results allowed us to localize the site of a procaine injection into the hippocampus, enabling us to begin to map tomographic reconstructions onto brain anatomy.