Simultaneous functional diffuse optical tomography and EEG in freely moving and anesthetized rats

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Introduction

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 readily 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

A DOT imager (NIRx Medical Technologies, LLC) constructed with two near-infrared lasers, 9 sources illuminated at 7Hz, and 16 detectors that detect light following source illumination was attached to an experimental setup (Fig 1) used for EEG recordings. 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 2). A plastic "Slinky" was used to suspend the tether removing its weight from the rat.

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. Following training, a DOT/EEG implant (Fig 2A) 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, placing the rat inside the cylinder and allowing it to forage for 15-20 minutes while DOT and EEG data were recorded (Fig 2B).

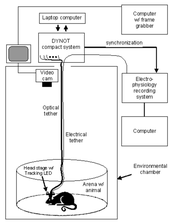

Figure 1: Schematic of experimental setup for rat foraging experiments.

Figure 2: Implant design. A) Implant (right) is affixed to the surface of the rat skull and interfaces with fiberoptic bundle and EEG tether via bolts (left). B) Foraging rat connected to fiberoptic bundle and EEG tether via headstage.

Methods - Foraging Data Analysis

EEG transitions into LIA (Fig 3, top) and theta (Fig 3, bottom) were detected using Matlab code. All LIA and theta epochs >4sec were identified and synchronized with DOT OxyHb and DeoxyHb signals based on time-stamps 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.

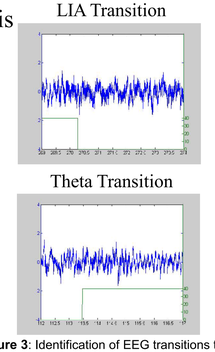

Figure 3: Identification of EEG transitions to LIA and theta. Representative EEG time series (blue) and ouput of program (green) designed to detect transitions to LIA (top) and theta (bottom).

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 4. OxyHb levels initially decrease in the 0-1sec interval after entering the EEG states (Fig 4, row 1), and increase dramatically during the interval 1-4sec after transition (Fig 4, row 4), in a way that resembles the Blood Oxygenation Level Dependent (BOLD) response observed with fMRI. In contrast, DeoxyHb initially increased after transition (Fig 4, row 2), and then decreased dramatically in the interval 1-4sec after transition (Fig 4, row 5). TotalHb did not change significantly following EEG transitions (Fig 4, rows 3 and 5), suggesting that blood volume remains relatively constant during EEG transitions.

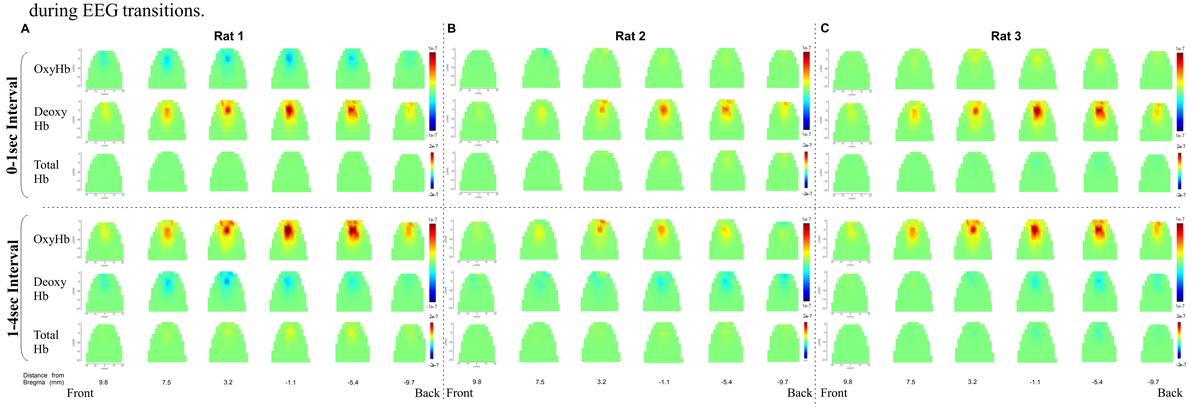

Figure 4: Temporal mean images highlight differences in the brain's hemodynamic state when EEG is in theta vs. LIA. Coronal brain slice images reconstructed for theta-LIA differences of OxyHb (rows 1 & 4), DeoxyHb (rows 2 & 5), and TotalHb (rows 3 & 6), over the intervals from 0-1sec (top panels) and 1-4 sec (bottom panels) after transition pairs are shown for all transitions averaged over a single session for three individual rats (A,B,C respectively). A BOLD-type response, characterized by an initial decrease in OxyHb levels in the 0-1sec interval after entering the EEG states (row 1) followed by a strong increase during 1-4sec after transition (row 4), was observed in nearly all sessions (20/23) for OxyHb. DeoxyHb levels showed an initial increase in the 0-1sec interval after transition (row 2), followed by a large decrease in the 1-4 sec interval after transition (row 5) in most sessions (21/23). TotalHb did not significantly or consistently change following EEG transition.

Results - Rat Foraging Experiments

Representative spatial mean time series (blue curves) plotted with their corresponding EEG transitions (green curves) are shown in Figure 5. OxyHb levels consistently change in a BOLD-like fashion upon EEG transition to theta, with an initial decrease followed by a gradual rise and plateau (Fig 5A, top), but not upon transition to LIA (Fig 5A, bottom), suggesting that the theta state is a higher energy state than LIA. DeoxyHb spatial mean time series changes opposite to OxyHb upon theta transitions (Fig 5B, left), with an initial increase in DeoxyHb levels followed by a decrease and plateau, but not upon transition into LIA. TotalHb spatial mean time series were found not to be linked to EEG state transitions (Fig 5B, right), suggesting that blood volume changes during transitions into theta and LIA were similar.

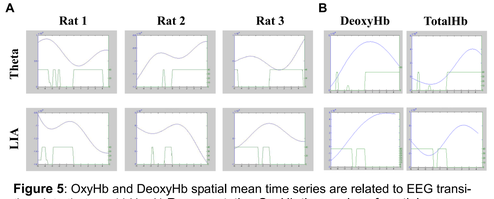

Figure 5: OxyHb and DeoxyHb spatial mean time series are related to EEG transitions into theta and LIA. A) Representative OxyHb time series of spatial means (blue) during transition into theta (top row) and LIA (bottom row) epochs on the same time scale as the theta score (green) for three individual rats. Upon transition to theta, OxyHb levels mimic the BOLD response observed with fMRI. B) Representative DeoxyHb (left column) and TotalHb (right column) time series for Rat 1.

Results - Rat Foraging "Transition Slope"

The "transition slope" was calculated as the slope of the line that runs through the points t=.5 sec and 2.5 sec after transition into theta or LIA in the spatial mean time series (Fig 6A). The transition slope was positive for OxyHb upon transition into theta but not LIA (Fig 6B), suggesting that the theta state is a higher energy state than LIA.

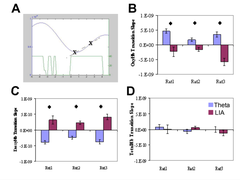

Figure 6: Transition slope calculation for transitions into theta and LIA. A) Transition slope calculation schematic. B) OxyHb had a positive transition slope upon theta transitions (blue bars), but not in LIA (red bars), across all rats (* p<0.0005). C) DeoxyHb had a negative transition slope following theta transitions, but not in LIA, across all rats (* p<0.0005). D) TotalHb had no discernable pattern related to EEG transitions. Error bars represent s.d.

Results - Rat Foraging "BOLD Correlation"

The "BOLD correlation" was calculated as the integral of the cross-correlogram between an idealized BOLD template and the spatial mean time series about the EEG transition (Fig 7A). The BOLD correlation was positive for OxyHb upon transition into theta but not LIA (Fig 7B), suggesting that theta transitions resemble the BOLD response. DeoxyHb spatial mean time series was compared with an idealized "reverse BOLD" template that was positive from 0-1sec and negative from 1-4sec (Fig 7C).

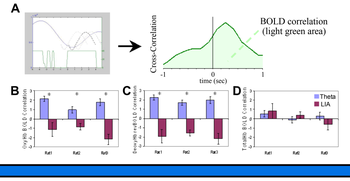

Figure 7: BOLD correlation calculation for transitions into theta and LIA. A) BOLD correlation calculation schematic. B) OxyHb levels had a positive BOLD correlation upon theta transitions (blue bars), but not in LIA (red bars), across all rats (* p<0.0005). C) DeoxyHb levels had a positive BOLD correlation with a "reverse BOLD" template following theta transitions, but not in LIA, across all rats (* p<0.0005). D) TotalHb had no discernable pattern related to EEG transitions. Error bars represent s.d.

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) and/or the putamen (+1.7mm infront of bregma; 3.0mm left of midline). Images were reconstructed and temporally averaged across 0-5min before, 5-10min after, and 60-65min after injection.

Results - Acute Procaine Injection

Injection of procaine caused a localized change in OxyHb distribution in the left hippocampus, such that there was less OxyHb present in the area of injection (black arrows) when compared to surrounding areas (Figure 8). All changes in OxyHb were consistent across all rats, suggesting that DOT is able to detect local metabolic brain changes.

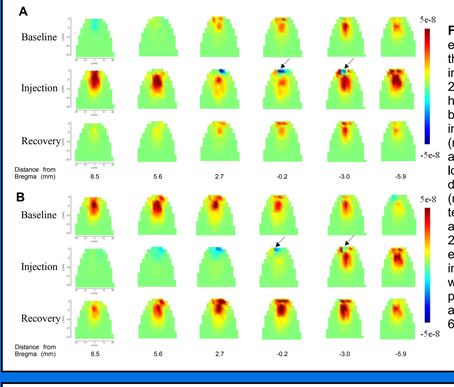

Figure 8: Procaine injection induces local hemodynamic changes in the brain. A) OxyHb temporal mean images for a single 1uL injection of 20% procaine into the left hippocampus, separated into baseline (top row) 0-5min before injection, 5-10min after injection (middle row) and recovery 60-65min after injection (bottom row). A localized decrease in OxyHb was detected around the site of injection (middle row, arrows). B) OxyHb temporal mean images preceding and following injection of 1uL of 20% procaine solution for a different rat. An asymmetrical decrease in OxyHb near the site of injection was again observed 5-10min after procaine injection (middle row, arrows), but was not detected 60-65min after injection (bottom row).

Results - Acute Procaine Injection Registration

An anterior (left putamen) injection of procaine caused a localized transient decrease in OxyHb distribution in the brain (Fig 9A,B). Horizontal sections averaged over the interval 15-16min after posterior (left hippocampal) and 5-6min after anterior injection (Fig 9C), and 25-26min after posterior and 15-16min after anterior injection (Fig 9D), show that DOT is able to monitor multiple sites in the brain simultaneously.

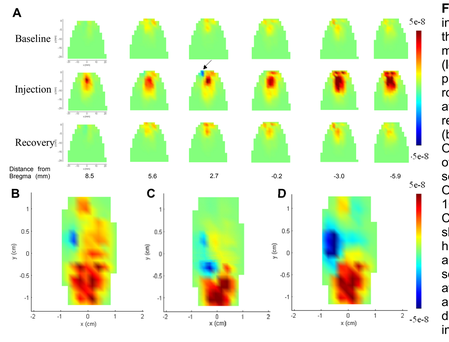

Figure 9: Dual procaine injections induce local hemodynamic changes in the brain. A) Coronal OxyHb temporal mean images for a single anterior (left putamen) 1uL injection of 20% procaine, separated into baseline (top row) 0-5min before injection, 5-10min after injection (middle row) and recovery 60-65min after injection (bottom row). A localized decrease in OxyHb was detected around the site of injection (arrow). B) Horizontal section (2.7mm below skull) of OxyHb temporal mean images 5-10min after anterior procaine injection. C) Horizontal section (2.7mm below skull) 15-16min after posterior (left hippocampal) and 5-6min after anterior injection. D) Horizontal section (2.7mm below skull) 25-26min after posterior and 15-16min after anterior injection. Time-varying decreases in OxyHb near sites of injection were observed.

Summary

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

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