

Non-invasive study of neurovascular coupling in awake, behaving monkey

Harsha Radhakrishnan, Helen Deng, Leeland Ekstrom, David A. Boas, Wim Vanduffel, and Maria Angela Franceschini
 MGH/MIT/HMS Athinoula A. Martinos Center for Biomedical Imaging, Charlestown, Massachusetts, 02129, USA

Our goal is to study neurovascular coupling non-invasively in awake, behaving monkeys.

- ✓ We used simultaneous scalp electroencephalography (EEG) and Near-Infrared Spectroscopy (NIRS) to measure the visual evoked potential (VEP), the hemodynamic and the fast optical responses to visual stimulation in two macaque monkeys.
- ✓ While the ability of NIRS to measure cerebral hemodynamic responses (slow optical signal) is well established, its ability to measure non-invasively the "fast optical signal" is still controversial. Here, we aim to determine the feasibility of performing NIRS measurements of the "fast optical signal" under optimal experimental conditions in awake behaving macaque monkeys.
- ✓ We also investigate the correlation between the VEP and hemodynamic responses in the visual cortex using full-field checkerboard with varying contrast.

methods

instruments

We optimized our optical instruments to acquire as fast as possible with optimal SNR (100-200Hz acquisition rate)

CW-CW4 system

- ✓ 2 co-localized laser diodes (690 nm and 830 nm, ~10 mW). Lasers always on. Frequency encoded (4.2 and 6 KHz modulation frequencies)
- ✓ 5 parallel APD detectors
- ✓ acquisition time after offline filtering: 10ms per data point
- ✓ 4 auxiliary channels record the stimulation trigger, pulse oximeter SaO₂, arterial pulsation, and IScan (recording eye position)

FD-ISS imager system

- ✓ 1 laser diode @ 830 nm (~3mW) always on
- ✓ 2 parallel PMT detectors
- ✓ acquisition time 4 ms per data point
- ✓ 4 auxiliary channels record the stimulation trigger, pulse oximeter SaO₂, arterial pulsation, and IScan (recording eye position)

EEG-Neuroscan NuAmps system

- ✓ We used 5 of 40 channels on a monopolar digital amplifier
- ✓ 1 electrode (4mm, Ag/AgCl) above peripheral V1
- ✓ 4 other electrodes (10mm, Ag/AgCl) - one on each ear (ground) and two on the forehead (reference and additional point of measurement)
- ✓ sampling rate is 1000 Hz
- ✓ 8 bit stimulus and 4 bit response inputs

probes

- ✓ 2 custom probes - ~1.7 cm diameter and ~2.5 cm length - were made with thermoplastic and held in place in the recording well using screws:
- FD probe: 1 source and 2 detectors at 1.5 cm from source.
- CW probe: 1 source and 5 detectors with the longest SD separation being 1.5 cm and the shortest separation being 0.6 cm
- 1 EEG electrode (4 mm, Ag/AgCl, Warner Instruments) was inserted in the middle of the CW probe.

CW probe schematic

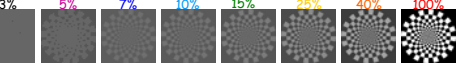
Positioning of the probe on the animal's head

animals and experimental setup

- 2 male macaque monkeys (*macaca mulatta*) (3-4 years old, 5-7 kg) M1 and M2
- Prior to our experiment:
 - plastic headset with post was surgically implanted on the skull
 - each monkey had a recording well (~1.8 cm diameter) surgically implanted above right peripheral V1, with the data exposed; proper post-operative care was provided
- After recovery, each monkey was trained to sit in the 'sphinx' position with the head restrained and to fixate to a small dot at the center of a contrast-reversing checkerboard
- Immediately before the optical measurements:
 - the monkey is seated in the chair
 - the probe is inserted and screwed to the recording well
 - additional EEG electrodes are attached to the head with EEG paste and tape
 - a pulse oximeter ear plug sensor is attached to one ear.
 - the monkey is placed in a dark chamber in front of a monitor and is given a juice reward for maintaining continuous fixation (2deg window)

stimulation protocols

- ✓ Each measurement session lasted 6-12 stimulation runs (6 min per run)
- ✓ Stimuli were presented on a screen (1024 x 768, 60Hz) at a distance of 46cm from the monkey's eyes
- ✓ We used full-field, contrast-reversing radial checkerboards as stimuli, in contrast with a uniform gray screen, with the same mean luminance, used for baseline stimulation.
- ✓ Fast signal (7 sessions with M1; 5 sessions with M2)
 - Block design 20 sec on and 20 sec off checkerboard (4, 7.5 Hz and random reversal frequencies)
 - Random epochs of reversing of radial checkerboard (250ms), ISI 0.2-3s
- ✓ Neurovascular coupling (5 sessions with both M1 and M2)
 - Event-related paradigm with 4 sec on contrast varying checkerboard interleaved with pseudorandom off periods (mean ISI of 12 s)
 - Contrast varying between:



Fast signal detection

VEP results

- ◆ Power spectra of the EEG data from the electrode in the recording well during representative runs on a 4 Hz stimulation (360 stimulus points) session on M2.
- ◆ While performing the FFTs we divided the data into two subsets, one considering the time intervals with stimulation on and one with stimulation off. The EEG response at the stimulation frequency is 15-20 dB higher when stimulation is on than when it is off and has strong harmonics.

We report only the results for the 4 Hz stimulation protocol. Results for the 7.5 Hz and random stimulation are consistent and very similar with the 4 Hz stimulation results

VEP responses obtained by averaging a representative single 6-min run (360 stimuli) during a session on M2. The error bars are standard errors.

Power Spectra of CW data

- ◆ Power spectra (left panel) of the CW & FD data before and after an adaptive heart filter (Franceschini and Boas, 2004).
- ◆ The adaptive filter strongly reduces the arterial pulsations in the 830 nm amplitude data.
- ◆ Because of the lower signal-to-noise at 690 nm and on the phase data the adaptive filter is less effective.

Slow optical signal results

- ◆ In each experiment, we verified that we could detect hemodynamic changes for every single 20-sec visual stimulus block
- ◆ Examples of slow vascular responses visible on the raw CW and FD data for each stimulation block. The data are bandpass filtered between 0.01 and 0.8 Hz but not block averaged.
- ◆ The CW and FD data segments in the figure were collected on M1 during two different sessions, stimulating for 20 s at 4 Hz.

Power Spectra of FD data

- ◆ The FFTs show no fast response peak at the stimulation frequency (4 Hz) or its harmonics in the traces with stimulation on.

For the 4-Hz CW we averaged 260 blocks; for the 7.5-Hz CW, and for the FD, we averaged 200 blocks.

Grand average of the slow optical responses (hemodynamic) for the CW (left) and FD (right) measurements. The error bars are standard errors. In the CW measurements the amplitude decreases at 830 nm is because of increased absorption at 830 nm (HbO increase), and the amplitude increases at 690 nm because of decreased absorption at 690 nm (HbR decrease). Similarly, in the FD measurements ac at 830 nm decreases and phase increases due to the absorption increase at that wavelength.

Fast optical signal results

- ◆ Grand average (10000 stimuli) of the fast amplitude and phase shift responses for the FD measurements. The error bars are standard errors.
- ◆ No significant changes with the fast signal periodicity are present.

Results for fast optical signals are averages of every second reversal of the checkerboard.

No changes in fast optical signal was found in each measurement in each monkey. We also tested the grand averages for the two monkeys, M1 and M2, separately and obtained similar results.

Monte Carlo simulations

To predict the magnitude of the effect of a focal scattering change within the brain cortex on our ac and phase measurements we performed Monte Carlo simulations on 3D structural MRI images of a macaque head (Boas et al., 2002). The Monte Carlo simulations also allowed us to evaluate the advantage of measuring cortical activation from the exposed dura, as compared to measuring such activation from the head surface.

Steinbrink et al. (Steinbrink et al., 2005) estimated that the scattering cortical changes associated with the fast signal are no larger than 0.4%.

The table report changes in ac and phase when above measurement noise level (ac standard errors ~0.005-0.01%, phase standard error ~0.001-0.002 deg after averaging hundreds of stimuli). Bold numbers are from the simulation, non bold are obtained extrapolating results to lower scattering changes

scattering % changes	subdura				head			
	15 min	20 min	25 min	30 min	10 min	15 min	20 min	25 min
0.1	0.9	2.4	0.8	0.4	4.9	3.8	1.2	2.1
0.1	0.1	0.2	0.2	0.2	0.5	0.4	0.5	0.4
0.4	0.1	0.1	0.1	0.1	0.2	0.2	0.2	0.2
0.4	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

From these simulations, a 0.2-0.4 % change in scattering produces an ac change above measurement noise in the well configuration, and below noise level if measured from the head surface

summary

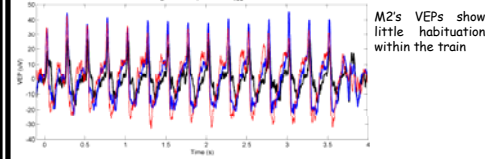
We performed semi-invasive simultaneous EEG and NIRS measurements to detect changes in fast optical signal and investigate neurovascular coupling in awake behaving macaque monkeys.

- ✓ Despite the large hemodynamic signal changes and strong VEP responses measured in every individual epoch, we could not detect any significant fast signal changes even after averaging thousands of stimuli across animals and sessions.
- These negative results in an optimized setting call into question the utility of such a small, elusive signal.
- ✓ Initial analysis of the VEP and hemoglobin responses during parametric stimulation show corresponding response amplitudes with varying contrast levels and saturation of both electrical and vascular responses at higher contrasts.

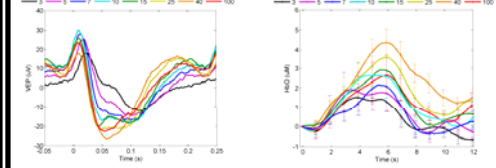
Neurovascular coupling: preliminary results

In a period of 4 months, we performed 5 measurement sessions on each monkeys and found that while the VEP responses in each animal were very consistent across sessions, the VEP responses of the two monkey were very different. Hence we did not average them together but report the results for each monkey separately.

M2 Grand average of the responses to stimulation train for 3 conditions

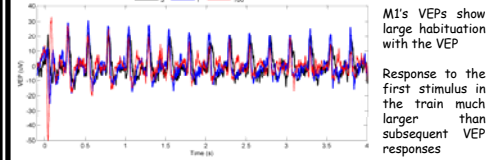


For each condition, VEP average of all stimuli

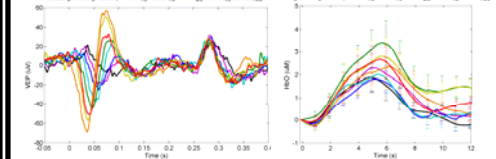


- ◆ For larger VEP responses, we observe larger HbO responses.
- ◆ Higher contrast do not produce larger VEP and HbO responses as both VEP and HbO saturate for higher contrasts.

M1 Grand average of the responses to stimulation train for 3 conditions



For each condition, response to first and second stimulus in the train



- ◆ Higher contrast do not produce larger VEP and HbO responses as both VEP and HbO saturate for higher contrasts.
- ◆ The correspondence between VEP and HbO for M1 is not as straightforward as for M2.

acknowledgments

Work supported by the US National Institutes of Health (NIH) grant R01-EB001954, R01-EB000790, R01-EY017081, the Human Frontiers Science Program (HFSP), GSKE, IUAP 5/04, and EF/05/014. The Martinos Center is supported by NCCR grant P41RR14075 and the MIND institute.

