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- we integrated scalp electroencephalography (EEG) and diffuse optical imaging (DOI) to investigate the neurovascular coupling in rats
- we modulated the SEP and hemodynamic responses to electrical forepaw stimulation either using parametric stimulation or pharmacologically by infusing g-aminobutyric acid (GABA) topically on the superficial cortical layers
- three SEP components (P1, N1, and P2) were used to predict the hemoglobin response

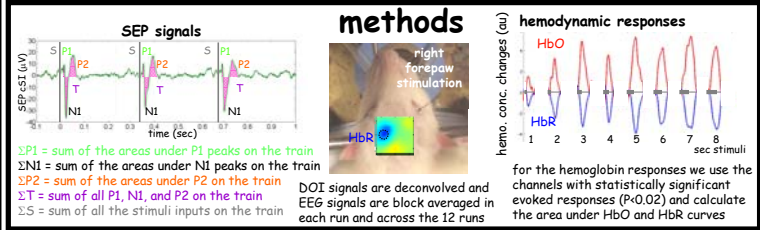
why looking at different SEP components?

different SEP components represent different populations of neurons which may control the hemodynamic response differently (i.e. excitatory or inhibitory) or to a different extent

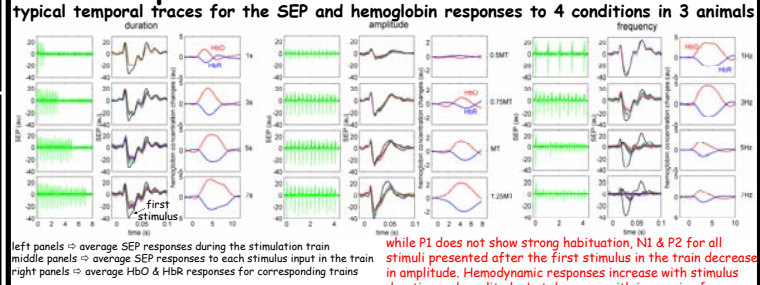
we wanted to test:

- whether the hemodynamic response is better predicted by a fixed input (stimulus inputs S) or by the SEP neuronal input
- to which SEP feature the DOI signal better correlates
- linearity and non-linearity of the hemodynamic and SEP responses
- whether the hemodynamic response is driven by postsynaptic activity in superficial or deep cortical layers

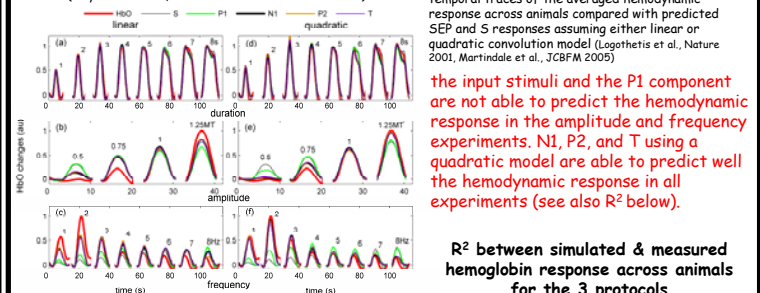
we found N1 and P2 to predict the hemoglobin response significantly better than P1



parametric stimulation results



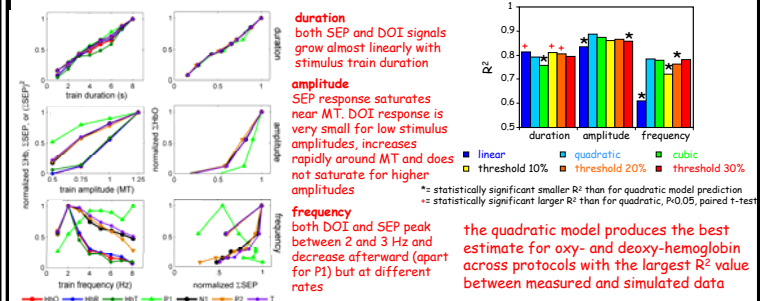
while P1 does not show strong habituation, N1 & P2 for all stimuli presented after the first stimulus in the train decrease in amplitude. Hemodynamic responses increase with stimulus duration and amplitude, but decrease with increasing frequency



The R² are the coefficients of determination calculated using the Fisher's Z transform to assess statistical significance we applied multifactor ANOVA to test the null hypothesis (no differences between R² values across the chromophores HbO, HbR, and HbT, and across animals) in the figure * = statistically significant smaller R² than for N1 prediction, P<0.05, paired t-test

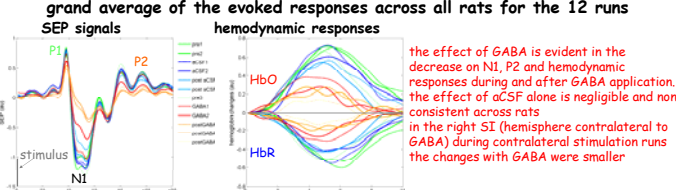
linearity & non-linearity of the responses

we used the integrated responses to assess the linearity of the hemoglobin response and the SEP features with respect to the stimulus conditions (left panels), and the linearity between the hemoglobin response and SEP features (right panels)

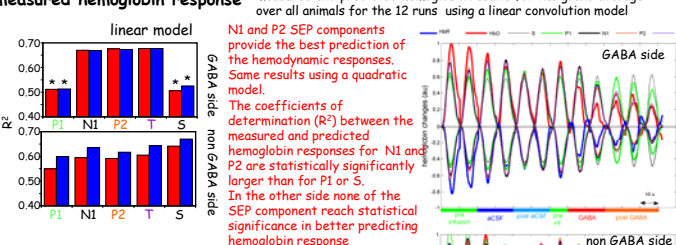


GABA experiment results

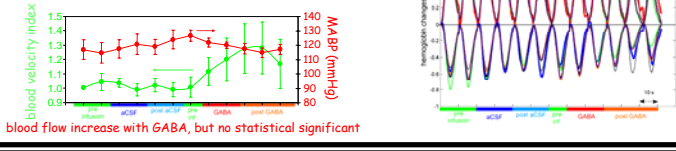
we modulate the synaptic activity during electrical forepaw stimulation pharmacologically by infusing g-aminobutyric acid (GABA) topically on the superficial cortical layers. GABA, the major inhibitory transmitter utilized by cortical neurons, reduces spike activity significantly by hyperpolarization. GABA does not cross the blood-brain barrier if injected intravenously, and does not penetrate into deeper cortical layers if applied topically on the brain surface (Roberts et al. Brain Res. 1980). It has also been shown to have different effects on the SEP components: In particular it enhances P1 and suppresses N1 (Brawley & Knight, Brain Res. 1984; Caesar et al., PNAS 2003; Staba et al. J Physiol. 2004). In our EEG measurements, we found similar behaviors of P1 and N1 with GABA. In addition, we observed a strong reduction of the hemodynamic response with GABA. These results not only further support our previous finding that P1 does not correlate with the hemodynamic response, but also suggest that the hemodynamic response is mostly driven by postsynaptic activity in superficial cortical layers.



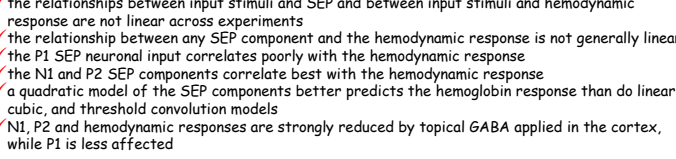
R² between simulated & measured hemoglobin response



prediction of the hemodynamic response



grand average of DCS regional blood velocity & blood pressure for the 12 runs



summary

- With these experiments we found:
 - the relationships between input stimuli and SEP and between input stimuli and hemodynamic response are not linear across experiments
 - the relationship between any SEP component and the hemodynamic response is not generally linear
 - the P1 SEP neuronal input correlates poorly with the hemodynamic response
 - the N1 and P2 SEP components correlate best with the hemodynamic response
 - a quadratic model of the SEP components better predicts the hemoglobin response than do linear, cubic, and threshold convolution models
 - N1, P2 and hemodynamic responses are strongly reduced by topical GABA applied in the cortex, while P1 is less affected
- The fact that topical GABA strongly reduce hemodynamic response suggest that the hemodynamic response is mostly driven by postsynaptic activity in superficial cortical layers (drug-induced effects on superficial neurons with secondary changes on deep cortical generators cannot be ruled out by these measurements)
- The finding that P1 does not correlate with the hemodynamic response while N1 and P2 correlate well with it suggests that the hemodynamic response is dominated by the populations of neurons associated with particular SEP components
- While we agree that it is critical to understand the differences in spatial sensitivity between the techniques used to measure the hemoglobin response and the neuronal activity, we found that the nonlinearity between the electrical and vascular responses persist, in our case where the volume averaged electrical and hemodynamic responses are considered
- These non-invasive measurements in rats can be directly translated to human measurements to study neurovascular coupling in humans

acknowledgments

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experimental setup

DOI system

- 18 laser diode sources (690 & 830 nm) frequency encoded
- 16 parallel APD detectors
- acquisition time per image 100ms
- detector's output digitized at ~40kHz on-line, individual source signals obtained off-line by infinite-impulse-response filters
- data analysis is done in Matlab and evoked hemodynamic responses are obtained
- 8 auxiliary channels record the stimulation trigger and physiological parameters such as blood pressure, pulse oximeter SaO₂, heart rate, respiration and end tidal CO₂

EEG system

- 40 channel monopolar digital amplifier
- sampling rate of 1000 Hz
- 8 bit stimulus and 4 bit response inputs
- event related potentials can be averaged and processed in real-time
- acquired data are exported to Matlab for subsequent analysis and determination of SEP components

DCS system (GABA exp.)

- 1 solid-state long coherence length laser @785 nm
- 4 photon-counting avalanche photodiodes
- intensity autocorrelation function computed by a digital correlator
- data are fitted off-line to a correlation diffusion model to obtain a blood velocity index

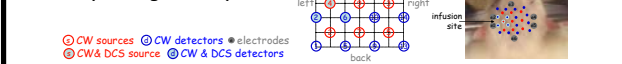
Neuroscan Labs
 http://www.neuro.com

Instrument replica of: T. Durduran, & A. G. Yodanis, Opt Lett, 2004

animal prep

- male Harlan Sprague-Dawley rats (weight 250-350g)
- anesthesia for surgery: gas mixture of 80% air & 20% O₂, and 1-3% isoflurane administered via face mask
- tracheotomy and cannulation of the femoral artery and vein
- ventilation parameters are adjusted to maintain PaCO₂ between 35 and 45 mmHg, PaO₂ between 140 and 180 mmHg, and pH between 7.35 and 7.45
- for the GABA experiment insertion of a catheter in the proximity of the left SI, & ventriculostomy immediately under the occipital bone for CSF drainage
- after surgery isoflurane discontinued; anesthesia maintained with a continuous intravenous infusion of α-chloralose of 40 mg/kg/hr
- heating blanket maintains core temperature of 37-38°C
- stimulation experiments are delayed by at least 1 hr to allow the anesthetic transition

probe geometry



experimental protocols

- left and right electrical forepaw stimulation
- 200 ms, >0.2 mA electrical stimuli delivered in trains
- 12 runs on each paw alternatively
- event related presentation with average ISA 12s

parametric stimulation

- changed train duration (1, 2, 3, 4, 5, 6, 7, 8 sec) (5 animals)
- changed train amplitude (0.5MT, 0.75MT, MT, 1.25MT) (9 animals) (motor threshold = MT)
- changed train frequency (1, 2, 3, 4, 5, 6, 7, 8 Hz) (5 animals)

GABA topical infusion (8 animals)

- for each run (12 total) we acquired: 2 min of DCW during rest; 4 min of CW and EEG during right forepaw stimulation (contralateral to GABA infusion site); 2 min of CW and EEG during left forepaw stimulation (ipsilateral to GABA site)
- stimulation parameters: 4s trains duration, 3Hz frequency and MT amplitude
- during infusion runs we continuously infused the cortex with a solution of artificial CSF (aCSF) alone, or with dissolved 1mM of GABA, at a rate of 0.02ml/hour