Analyzing Trial-to-Trial Variability in the Mouse Visual Pathway Using Functional Ultrasound

Erol, Aybuke; Kruizinga, Pieter; Hunyadi, Borbala

DOI
10.1109/ISBI56570.2024.10635718

Publication date
2024

Document Version
Final published version

Published in
IEEE International Symposium on Biomedical Imaging, ISBI 2024 - Conference Proceedings

ANALYZING TRIAL-TO-TRIAL VARIABILITY IN THE MOUSE VISUAL PATHWAY USING FUNCTIONAL ULTRASOUND

Aybüke Erol[1], *Pieter Kruizinga*[2], *Borbála Hunyadi*[1]

[1]Signal Processing Systems (SPS), Delft University of Technology, Delft, The Netherlands
[2]CUBE, Department of Neuroscience, Erasmus Medical Center, Rotterdam, The Netherlands

2024 IEEE International Symposium on Biomedical Imaging (ISBI) | 979-8-3503-1333-8/24/$31.00 ©2024 IEEE | DOI: 10.1109/ISBI56570.2024.10635718

ABSTRACT

Functional ultrasound (fUS) is an emerging neuroimaging modality that records changes in local blood dynamics. While it is known that the brain can respond variably to the same stimuli presented at different time instants, the extent to which fUS detects this variability based on the measured hemodynamics remains an open question. In this work, we characterize trial variability using fUS by estimating activation coefficients per trial using region-specific hemodynamic response functions. Our visual fUS experiments conducted on a mouse consistently reveal an increase of trial variability from the lateral geniculate nucleus to the visual cortex across different brain slices. These results are in parallel with prior findings in neuronal studies, suggesting a link between fluctuations of the evoked fUS response and true neural variability.

***Index Terms*—** hemodynamic response, trial variability, functional ultrasound

1. INTRODUCTION

Functional ultrasound (fUS) is a relatively new neuroimaging technique that, similar to functional magnetic resonance imaging (fMRI), measures hemodynamics as a proxy for neural activity [1]. Both modalities frequently employ multiple repetitions (*trials*) of stimuli in order to improve the signal-to-noise ratio (SNR) for capturing evoked activity and enhance the statistical power of the study [2]. The majority of fUS and fMRI analyses use the time courses of stimuli to extract spatial activation maps, either via linear regression (known as the general linear model - GLM) or by computing the per-voxel Pearson correlation coefficient (PCC). For both approaches, the stimulus time course, represented as a boxcar function indicating when the stimulus is on or off, is convolved with a hemodynamic response function (HRF).

An inherent assumption of using the convolutional model is that the brain response is time-invariant. However, we know this assumption does not hold due to various neurobiological mechanisms such as neuronal adaptation, stimulus expectation, time-varying functional connectivity, fluctuating attention or simply from noise [3]. When this variability is not taken into account, trial-averaging can lead to an imprecise portrayal of the brain's actual response. In more extreme scenarios, this imprecision could potentially yield erroneous conclusions in neurocognitive research [4].

In the literature, there are several approaches proposed for tackling trial variability. For example, a common procedure is *selective* averaging of trials by rejecting outlier trials. To determine which trials should be considered as unreliable, various methods have been employed such as amplitude-based thresholding (attributed to motion or eye blink artifacts) [5, 6] or visual inspection [7]. Nevertheless, experiments have shown that repeating of stimuli not only induces changes in the magnitude, but also in the peak latency or duration [8] of individual trial responses. As such, only tracking the changes in magnitude may not be adequate to represent the full extent of trial variability.

The GLM framework has also been used to model trial variability, for instance, by defining a separate regressor for each trial, or picking a trial of interest and grouping every other trial under another regressor [9]. Allowing for trial variability was shown to improve classification accuracy with fMRI [10]. However, it's important to note that these methods employ a fixed HRF in their designs, despite the known variations in HRF across individuals and brain regions [11]. In our work, we define the data matrix with columns corresponding to distinct trial responses of a voxel. Likewise, we characterize the model time course for a single trial response, i.e. we convolve a voxel-specific HRF with one stimulus epoch. Finally, we estimate trial activation coefficients for each region of interest (ROI) using linear regression.

Despite the increasing utilization of fUS as a new imaging technique for both neuroscientific and clinical applications [12], owing to its portability, low cost and high spatiotemporal resolution, trial variability as detected by fUS has not yet been explored. In our analysis, we focus on the fUS responses of two crucial ROIs of the mouse brain's visual-processing pathway, the lateral geniculate nucleus (LGN) and visual cortex (VIS), and examine their temporal dynamics through trial variability.

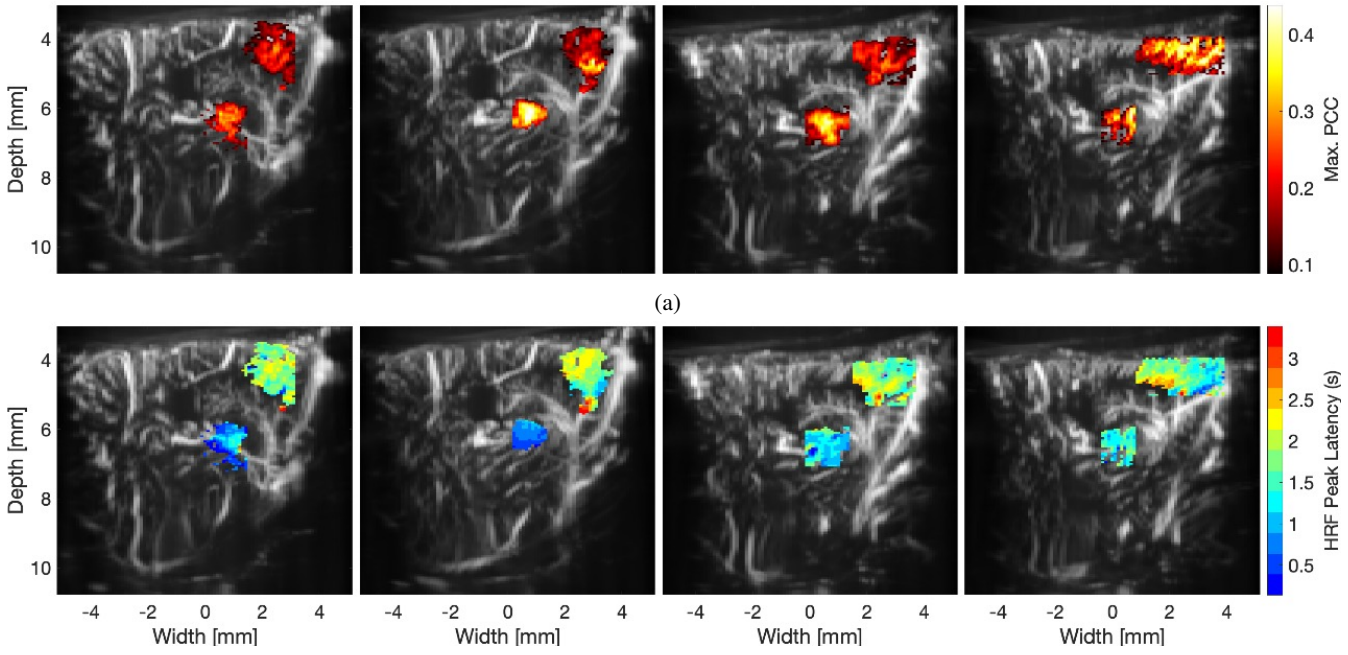


Fig. 1: Thresholded correlation images (a) obtained using the optimal HRF peak latency (b), overlaid against the mean PDI, displaying LGN (bottom region) and VIS (top region). The slices were imaged at Lateral +2.55, -2,55, +2.15 and -2.15 mm from left to right respectively.

2. METHOD

For each voxel, we segment the observed response into trials, and place these segments to columns of a matrix Y. We define our data model as:

$$Y = X\beta + \epsilon, \tag{1}$$

where $Y \in \mathbb{R}^{T\times N}$ is the data matrix, $\beta \in \mathbb{R}^{K\times N}$ is the matrix of activation coefficients, $X \in \mathbb{R}^{T\times K}$ contains the design variable(s), $\epsilon \in \mathbb{R}^{T\times N}$ represents noise, N is the number of stimulus repetitions, T is the number of time samples included within one trial and K is the number of different types of stimuli. The proposed method is illustrated over example simulated data in Fig. 2. Note that, we employed a single type of visual stimulus in our fUS experiments, hence for consistency the illustration is given for $K = 1$. It is possible to incorporate different stimuli by including their responses together in the trial segments. Then, a separate regressor can be defined with the onset of each event, as a new column in X.

For constructing the design variable, we estimated voxel-specific HRFs by shifting the HRF peak latency at various values using a single gamma function [13]: $h(t, \boldsymbol{\theta}) = \Gamma(\theta_1)^{-1}\theta_2^{\theta_1} t^{\theta_1-1}\mathrm{e}^{-\theta_2 t}$. We determined the HRF of a voxel as the one that provides the highest PCC with the stimulus across slices. Finally, we estimate $\hat{\beta} = X^{\dagger}Y$ where $(.)^{\dagger}$ is the pseudo-inverse. We aimed at answering the following research questions:

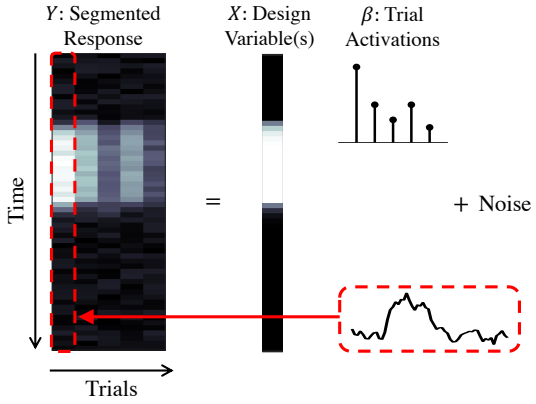


Fig. 2: Depiction of the proposed method for $K = 1$. Each column of Y stands for a trial response. The design variable is given by the convolution of a stimulus trial with an HRF.

Q1. Do the trial activations stay constant, or do they vary during the experiment? If they do, is this variation random or could we track a trend?

Q2. Do the variations occur simultaneously at the ROIs?

Q3. Can we find a reproducible difference in variability between LGN and VIS across slice recordings?

To unveil the trend of trial activations, we fit a linear model to the estimated β coefficients of each ROI (Q1). Sub-

sequently, we express the trial activations of VIS as a function of those of LGN to see if they vary in a correlated manner (Q2). Finally, we compute the coefficient of variation (CoV) of the trial activations of the ROIs (Q3).

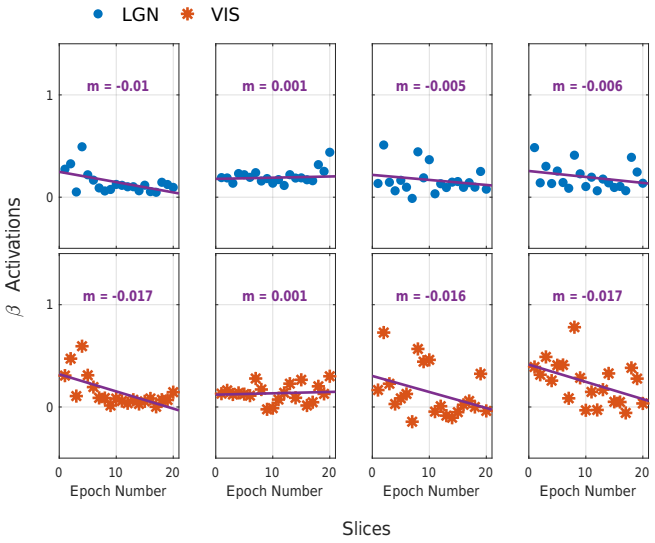
3. EXPERIMENTAL SETUP

Functional ultrasound records the hemodynamic activity of the brain in 2D slices using ultrafast Doppler imaging. Imaging was performed on an awake head-fixed mouse (7-months old male, C57BL/6J), by transmitting 20 tilted plane waves ($\pm 8°$) through a cranial window covered by a TPX layer, while the mouse was able to freely walk on a rotating wheel. After Fourier-domain beamforming and angular compounding of the echo waves, singular value decomposition-based clutter filtering [14] was utilized to remove tissue components. We worked on power-Doppler images (PDIs) sampled at a rate of 4 Hz. The mouse brain was imaged sagittaly at 4 locations (Lateral $\pm 2.15, \pm 2.55$ mm), all of which captured LGN and VIS (Fig. 1). The visual stimulus consisted of randomly generated high contrast images (white speckles against a black background). The stimulus was repeated 20 times throughout the experiment and its duration was kept at 4 s, while the rest periods in-between stimuli were randomized from 10 to 15 s. We applied spatial smoothing using a Gaussian kernel with a standard deviation of 0.5 voxels in size, and standardized the voxel time-series to zero-mean and unit variance prior to further processing.

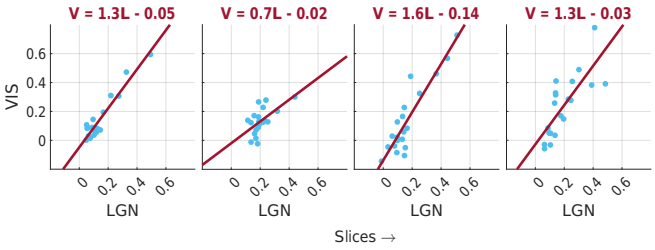
To determine significantly active voxels for each ROI, we selected a P-value threshold of 0.0001 and converted this value to z-score using a two-tailed test (z-score: 3.71). Next, we applied Fisher's transform to this z-score and arrived at a PCC threshold of $c = 0.1$ [15]. We calculated the PCC of each voxel by correlating the voxel time-series with the HRF-convolved stimulus time course. The thresholded correlation images are displayed in Fig. 1. We segmented the voxel time-series into trials that start 3 s before a stimulus onset and ends 12 s after. Note that, these values are determined in accordance with our stimulus design, but can be re-adjusted based on the paradigm. All segments were baseline corrected by subtracting the mean amplitude of the pre-stimulus response ([-3,0] s with respect to the stimulus onset time).

4. RESULTS

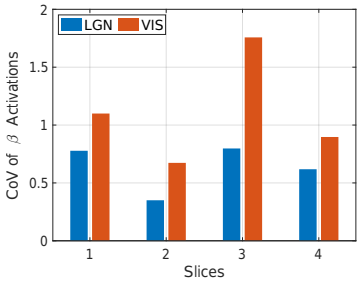
Using the optimal HRFs, we estimated trial activations per voxel. We averaged the trial activations across the voxels of a ROI at each slice. The results are provided in Fig. 3(a). To start with, we observe a declining trend of trial activations as the trials progress. As discussed in the Introduction, many factors including neuronal adaptation or habituation can cause such descent. For studying the correlation of the timing of these variations between LGN and VIS, we plotted the trial activations of VIS as a function of those of LGN. The link between the two regions can be clearly observed in Fig. 3(b), as the best-fitting line shares similar parameters (slope and intersection) across slices. Note that, this correlation is expected, at least up to some degree, as VIS is anatomically connected to, and receives direct input from the LGN.



(a)



(b)



(c)

Fig. 3: (a) Variation of β across trials for each region and brain slice. We plotted the best-fitting line (in least-squares sense) to β values in each case to highlight the trend of activations across trials. The slope m of the predicted line is indicated on top of each plot. (b) Trial activations of LGN vs. VIS. The equation written on each plot describes the line of best fit in least-squares sense (shown in red color) between VIS and LGN, denoted by V and L respectively. (c) Coefficient of variation (CoV) of β across regions and slices.

Another point worth discussing is the offset in the line equations relating LGN to VIS (Fig. 3(b)). This offset is caused by the occasional negative activations of VIS, albeit small both in absolute value and quantity. This negativity comes as a result of VIS amplitudes dropping below the baseline in certain trials. We can consider the possibility that the mouse was not responsive in those trials, and the effects of noise (physiological and/or instrumentation related) became too dominant. However, in those same trials, negative activations were almost never found in LGN. Indeed, stimulus-evoked negative activations in VIS were reported before with fMRI as well [16]. For both Fig. 3(a) and (b), the results of the second slice are slightly different from the rest. Although this difference might be due to the particular slice that was imaged or a change in the mouse's attention, it is worth to mention that this slice has the least amount of active voxels, possibly affecting it's generalizability compared to the rest.

In order to assess the overall variability of the two regions, we calculated the CoV of β's, defined as the ratio of the standard deviation to the mean (Fig. 3(c)). We can observe that VIS exhibits a variability that is twice as high as that of LGN for all slices, in accordance with neuronal findings [17]. In fact, the transformation between LGN and VIS is argued to be the point at which the large response variability of VIS originates [18]. As a last note, we observed that the PCC values calculated conventionally (i.e., via correlating the whole HRF-convolved stimulus time course with the voxel time-series, Fig. 1(a)) share a very similar trend with the summation of trial activation coefficients across voxels. On the contrary, the CoV of trial activations follows an opposing trend (Fig. 4), indicating that PCC values might drop as a result of higher variability.

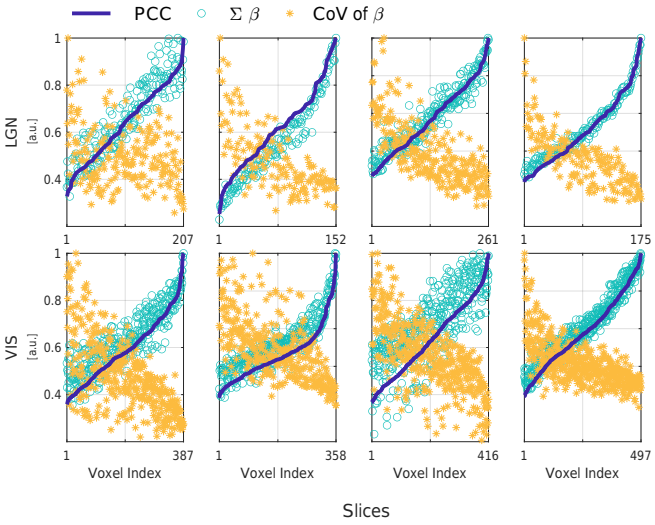


Fig. 4: Change of PCC values, summation of non-negative trial activations and CoV of trial activations across voxels. Note that, the voxels were sorted in ascending PCC and all measures were normalized to have a maximum amplitude of 1 for easier interpretability of the plots.

5. CONCLUSION

In this study, our objective was to investigate trial variability using fUS. By segmenting the observed data into individual trial responses, we computed activation coefficients for each trial within specific regions of interest. We noted a general decline in activations throughout the experiment, potentially influenced by intricate brain mechanisms such as neuronal adaptation. Our comparison of trial variability between LGN and VIS revealed higher variability in the cortex. This consistent difference in variability across all our recordings aligns with established neuronal findings. Our results imply a potential link between the variability of fUS responses and the underlying neuronal variability. Trial variability can be assessed via different metrics other than an HRF-based model-fitting as proposed in this paper, such as by tracking the changes in signal power or amplitude across trials. In the future, such methods can be compared for evaluating trial variability, while the temporal dynamics of LGN and VIS should be further validated with more experiments performed over multiple subjects.

6. COMPLIANCE WITH ETHICAL STANDARDS

All experimental procedures were approved *a priori* by an independent animal ethical committee (DEC-Consult, Soest, the Netherlands), and were performed in accordance with the ethical guidelines as required by Dutch law and legislation on animal experimentation, as well as the relevant institutional regulations of Erasmus University Medical Center.

7. ACKNOWLEDGEMENT

This study was funded by the Synergy Grant of Department of Microelectronics of Delft University of Technology and the Delft Technology Fellowship.

8. REFERENCES

[1] T. Deffieux, C. Demené, and M. Tanter, "Functional ultrasound imaging: A new imaging modality for neuroscience," *Neuroscience*, vol. 474, pp. 110–121, 2021.

[2] W. Yan and Y. Wu, "A time-frequency denoising method for single-channel event-related EEG," *Frontiers in Neuroscience*, vol. 16, pp. 991136, 2022.

[3] G. Ouyang and C. Zhou, "Characterizing the brain's dynamical response from scalp-level neural electrical signals: a review of methodology development," *Cognitive Neurodynamics*, vol. 14, no. 6, pp. 731–742, 2020.

[4] G. Ouyang, W. Sommer, and C. Zhou, "Reconstructing ERP amplitude effects after compensating for trial-to-trial latency jitter: a solution based on a novel application of residue iteration decomposition," *International Journal of Psychophysiology*, vol. 109, pp. 9–20, 2016.

[5] É. Macé, G. Montaldo, S. Trenholm, C. Cowan, A. Brignall, A. Urban, and B. Roska, "Whole-brain functional ultrasound imaging reveals brain modules for visuomotor integration," *Neuron*, vol. 100, no. 5, pp. 1241–1251, 2018.

[6] F. Moradi, L. Liu, K. Cheng, R. A. Waggoner, K. Tanaka, and A. A. Ioannides, "Consistent and precise localization of brain activity in human primary visual cortex by MEG and fMRI," *Neuroimage*, vol. 18, no. 3, pp. 595–609, 2003.

[7] K. J. Mullinger, M. T. Cherukara, R. B. Buxton, S. T. Francis, and S. D. Mayhew, "Post-stimulus fmri and eeg responses: evidence for a neuronal origin hypothesised to be inhibitory," *Neuroimage*, vol. 157, pp. 388–399, 2017.

[8] M. W. Guy, S. Conte, A. Bursalıoğlu, and J. E. Richards, "Peak selection and latency jitter correction in developmental event-related potentials," *Developmental psychobiology*, vol. 63, no. 7, pp. e22193, 2021.

[9] H. Abdulrahman and R. N. Henson, "Effect of trial-to-trial variability on optimal event-related fmri design: Implications for beta-series correlation and multi-voxel pattern analysis," *NeuroImage*, vol. 125, pp. 756–766, 2016.

[10] J. A. Mumford, B. O. Turner, F. G. Ashby, and R. A. Poldrack, "Deconvolving bold activation in event-related designs for multivoxel pattern classification analyses," *Neuroimage*, vol. 59, no. 3, pp. 2636–2643, 2012.

[11] D. A. Handwerker, J. M. Ollinger, and M. D'Esposito, "Variation of BOLD hemodynamic responses across subjects and brain regions and their effects on statistical analyses," *Neuroimage*, vol. 21, no. 4, pp. 1639–1651, 2004.

[12] S. Soloukey, A. J. Vincent, D. D. Satoer, F. Mastik, M. Smits, C. M. Dirven, C. Strydis, J. G. Bosch, A. F. van der Steen, C. I. De Zeeuw, et al., "Functional ultrasound (fUS) during awake brain surgery: the clinical potential of intra-operative functional and vascular brain mapping," *Frontiers in neuroscience*, vol. 13, pp. 1384, 2020.

[13] A. Erol, C. Soloukey, B. Generowicz, N. Van Dorp, S. Koekkoek, P. Kruizinga, and B. Hunyadi, "Deconvolution of the functional ultrasound response in the mouse visual pathway using block-term decomposition," *Neuroinformatics*, vol. 21, no. 2, pp. 247–265, 2023.

[14] C. Demené, T. Deffieux, M. Pernot, B.-F. Osmanski, V. Biran, J.-L. Gennisson, L.-A. Sieu, A. Bergel, S. Franqui, J.-M. Correas, et al., "Spatiotemporal clutter filtering of ultrafast ultrasound data highly increases doppler and fultrasound sensitivity," *IEEE transactions on medical imaging*, vol. 34, no. 11, pp. 2271–2285, 2015.

[15] C. Brunner, M. Grillet, A. Urban, B. Roska, G. Montaldo, and E. Macé, "Whole-brain functional ultrasound imaging in awake head-fixed mice," *Nature Protocols*, vol. 16, no. 7, pp. 3547–3571, 2021.

[16] D. Bressler, N. Spotswood, and D. Whitney, "Negative bold fmri response in the visual cortex carries precise stimulus-specific information," *PLoS One*, vol. 2, no. 5, pp. e410, 2007.

[17] P. Kara, P. Reinagel, and R. C. Reid, "Low response variability in simultaneously recorded retinal, thalamic, and cortical neurons," *Neuron*, vol. 27, no. 3, pp. 635–646, 2000.

[18] M. Gur, A. Beylin, and D. M. Snodderly, "Response variability of neurons in primary visual cortex (V1) of alert monkeys," *Journal of Neuroscience*, vol. 17, no. 8, pp. 2914–2920, 1997.