Discussion of Signal Averaging in Direct Imaging of Neuronal Activity (DIANA) Data Analysis

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A novel fMRI method called DIANA (Direct Imaging of Neuronal Activity), which allows direct detection of neuronal activity and its propagation in somatosensory networks, was recently reported using anesthetized mice at 9.4 T with 5ms temporal resolution[1]. Unfortunately, difficulty in replicating the DIANA signal has also been reported[2,3]. While there may be several reasons worth discussing in this regard, here we discuss one of them specifically related to signal averaging in DIANA data analysis. This pertains to the proposed direct correlation between DIANA and neuronal activity which may profoundly affect the improvement of the sensitivity of DIANA responses through signal averaging.

According to our observation made through DIANA data analysis, averaging the same number of trials across mice provides better sensitivity of the DIANA responses than averaging trials in a single mouse, and averaging more trials may not guarantee a higher sensitivity of the DIANA response. This observation appears to conflict with the general principle of fMRI that signal averaging enhances the sensitivity of BOLD responses. Typically, signal averaging improves the signal-to-noise ratio (SNR), usually calculated by dividing the mean of the main signal by the standard deviation (SD) of the background noise. For example, summing up N repeated measurements increases the main signal by N, increases the SD of the noise by $\sqrt{N}$, and therefore increases SNR by $\sqrt{N}$. However, this well-known principle is based on two fundamental assumptions: the consistency of the main signal and the randomness of the noise. In terms of the consistency of the main signal, DIANA has been proposed to exhibit direct correlation with neural activity, and the percent change of the DIANA response is relatively small (~0.1-0.2%), so the variability of neural responses[4], including neural adaptation[5], should be more seriously considered in data acquisition and analysis (compared to conventional BOLD-fMRI). Additionally, the randomness of noise, such as baseline fluctuation, needs to be evaluated. To investigate the randomness of baseline fluctuation and compare the averaging of within-subject and between-subject data, varying number of trials were randomly selected from a random single mouse, and for comparison, an equal number of trials were randomly selected from each of 5 mice at 11.7 T[6] and 9.4 T[1] (Fig.1). The SD of the pre-stimulation period was then calculated and averaged across 100k iterations. As shown in Fig. 2, the baseline fluctuation was reduced more effectively (i.e., better approaching the $1/\sqrt{N}$ line) via between-subject averaging than via within-subject averaging in both 11.7T (A)[6] and 9.4T DIANA data (B)[1]. The fact that the SD reduction of the within-subject averaging deviates from the $1/\sqrt{N}$ line suggests that baseline fluctuations in the DIANA signal have a pseudo-random signal component, which probably originates from spontaneous neural activity[4]. The better suppression of baseline fluctuations in between-subject averaging may be due to unique frequency and phase of neural oscillations in the brain network[9]. To test the hypothesis, we also performed simulations by generating artificial signals consisting of deterministic neural responses, oscillations of a specific frequency and phase range, and random noise, and the results showed similar trends to the in vivo data (Fig. 2C). Taken together, to ensure good identification of DIANA responses, it is practically recommended to minimize significant neural adaptation (e.g., 30 s rest every two or three trials) and average data across subjects until an effective data analysis pipeline is established.

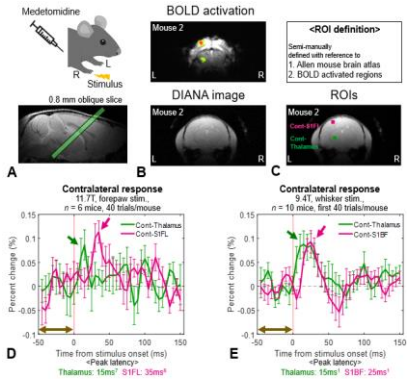

Figure 1. (A) Illustration of DIANA experiment using forelimb electrical stimulation in mice anesthetized with medetomidine (top), acquiring images from a single oblique slice containing the thalamus and S1FL areas (bottom). **(B)** BOLD activation map ($p < 0.05$, top) and DIANA image (bottom) from a representative mouse. **(C)** Regions of interest (ROIs) for extracting time series. **(D)** Averaged DIANA time series at 11.7T[6] and **(E)** 9.4T[1] for reference.

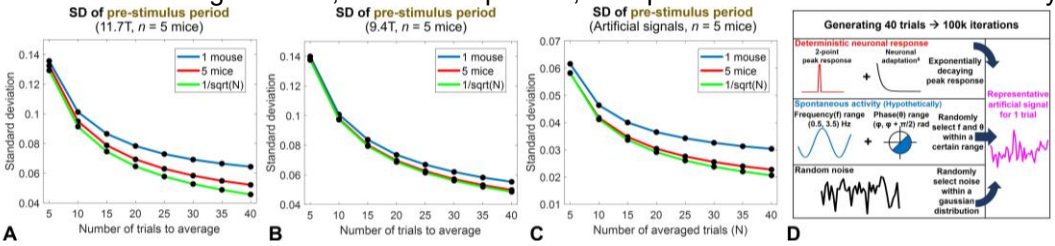

Figure 2. Standard deviation (SD) of pre-stimulus period was calculated with respect to the number of averaged trials (N) using **(A)** 11.7T[6], **(B)** 9.4T[1] and **(C)** artificial data. **(D)** Composition of the artificial signals. The 5 mice were randomly selected for each repetition (5 is a divisor of 40).

References: [1] Toi et al. *Science* 378 (2022). [2] Choi et al. *bioRxiv* (2023). [3] Hodono et al. *Imaging Neuroscience* 1 (2023). [4] Amos Arieli et al. *Science* 273 (1996). [5] Benda, J. *Current Biology* 31 (2021). [6] Toi et al. *ICMRI* (2023). [7] Sanganahalli et al. *J. C. BI. Flow Metab.* (2016). [8] Thanh Tan Vo et al. *PNAS* (2023). [9] Buzsáki et al. *Science* 304 (2004).