Direct Imaging of Neuronal Activity (DIANA) fMRI in mice at 7 T without trigger-related delay

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Introduction: Two years ago, our group reported a novel functional MRI (fMRI) method called direct imaging of neuronal activity (DIANA), enabling direct detection of neural activity on millisecond timescales [1]. However, there are criticisms that the DIANA signal may be an artifact signal due to nonideal aspects of the pulse sequence. Phi Van et al. reported that the delay assigned to trigger stimulation in the DIANA pulse sequence can disrupt the steady state of the spin system and cause undesired signals that are not related to neuronal activation [2]. They made the important discovery that even small trigger delays (e.g., 12 μs) in the line-scan pulse sequences of DIANA fMRI can produce unwanted signals. In this study, we report that the DIANA fMRI can capture *in vivo* neural activity during forepaw stimulation without trigger-related delay.

Methods

Experiment: All experiments were performed using a volume coil (72mm/112mm) for RF transmission and a 2×2 surface array coil for signal reception on a 7T animal scanner (BioSpec, Bruker). (i) *Phantom imaging.* Experiments were performed using a modified version of the line-scan imaging sequence used by Toi et al [1]. To change the trigger-related delay, we modified the delay assigned to the trigger signal (TTL1_LOW) in Bruker ppg file (**Fig.1A**). To make the trigger-related delay zero, trigger signals, which were previously located outside the repetition time (TR) loop, have been moved inside the TR loop. (**Fig.1B**). A saline phantom was prepared and scanned with various trigger-related delays (i.e., 0us, 6us, 17us, 55us). 50 trials were acquired for each condition. Scan parameters were as follows: TR/TE, 5/2ms; flip angle, 4°; field of view, 16×16mm^2; matrix size, 72×72; and slice thickness, 1.0mm. Sufficient dummy scans (10s, 2000TR) were used to achieve steady-state magnetization prior to the main sequence. Both gradient and RF spoiling were used to suppress the residual transverse magnetization.

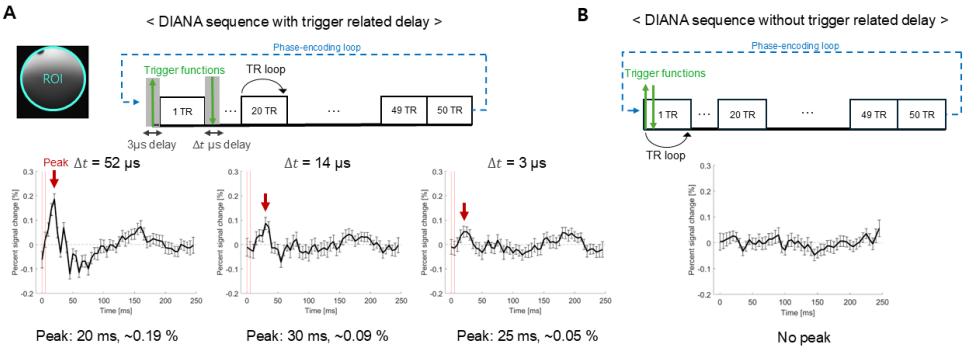

Figure 1. (A) Illustration of the DIANA sequence with trigger-related delays for varying trigger delays (i.e., 55 μs, 17 μs, and 6 μs). The green upward-arrow indicates 'TTL1_HIGH', which turns the trigger on (or off), and downward-arrow indicates 'TTL1_LOW', which turns the trigger off (or on). In the phantom experiment, time series with 50 trials averaged. The vertical red line indicates the timing when the delay exists. **(B)** The modified DIANA fMRI sequence without trigger-related delays.

(ii) *DIANA fMRI.* Twelve adult ketamine/xylazine-anesthetized Cre-inducible DTR transgenic mice (iDTR) mice were used. Two coronal slices covering the thalamus and forelimb primary somatosensory cortex (S1FL), respectively, were acquired using electrical forelimb stimulation (**Fig.2A**). The sequence without trigger-related delay was used for DIANA fMRI (**Fig.2B**). The same scan parameters as for phantom imaging were used. Electrical stimulation (current, 0.5mA; duration, 1ms; frequency, 4Hz) was delivered to the right forepaw. The stimulus was repeated 72 times, equal to the phase-encoding steps, with an interval of 250ms. The stimulation paradigm consisted of 100ms pre-stimulation, 1ms stimulation, and 149ms post-stimulation. 40 trials per mous e were acquired and used for analysis. To minimize neural adaptation, a 90-second rest period was provided every 5 trials.

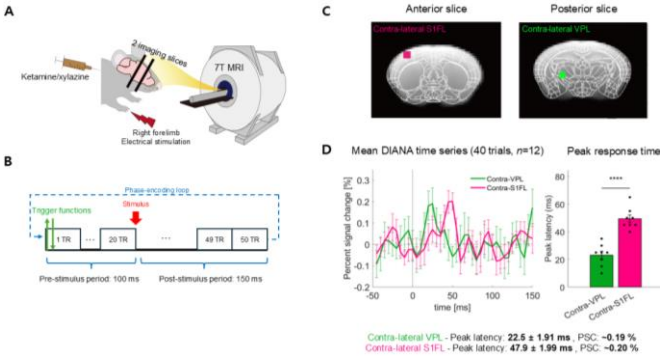

Figure 2. (A) Schematic of the experimental setup **(B)** Schematic of the modified DIANA sequence without any delay. Electrical stimulation was applied 100ms after the trigger signal, which is at the beginning of the phase-encoding loop. **(C)** 3-by-3 square ROIs of Contra-lateral S1FL and VPL, in the anterior and posterior slices, respectively. **(D)** Mean DIANA response and peak response time in VPL and S1FL. Data represent mean ± SEM. ****: $p < 0.0001$ for one-tailed Wilcoxon signed rank test

Data analysis: For group analysis, all DIANA images were linearly registered to the image of mouse #1. For the ventral posterolateral nucleus (VPL) of the thalamus and S1FL, a 3×3 square region of interest was defined with reference to the Allen Mouse Brain Atlas (**Fig.2C**). Time series were extracted from each ROI, using temporal smoothing (3-point Gaussian kernel), ROI averaging, signal normalization, and linear detrending. All 40 trials per mouse were averaged and they were averaged across all 12 mice. For phantom imaging, time series were extracted from the entire region of the phantom, averaging all 50 trials.

Results: The DIANA signals acquired using the sequences with different trigger-related delays were shown in **Fig.1**. When using a saline phantom with a delay of 55μs, a signal change with a peak of ~0.19% was observed 20ms after trigger onset, and it decreased with decreasing delay. After moving the trigger functions inside the TR loop (**Fig.1B,** top), we confirmed that the signal change due to the trigger-related delay was well suppressed (**Fig.1B,** bottom). Using the sequence without trigger-related delay, we identified the thalamocortical (TC) pathway from the VPL to S1FL (**Fig.2D**, left). In response to the electrical forelimb stimulation, statistically significant DIANA responses were sequentially observed in the VPL and S1FL with peak latencies of 22.5±1.91 ms (~0.19%) and 47.9±1.99 ms (~0.20%), respectively (n = 12, **Fig.2D**, right).

Discussion and Conclusion: In this study, we modified the sequence program to make the trigger-related delay zero, and without trigger-related delay, we were still able to sequentially detect DIANA responses in the VPL and S1FL during electrical stimulation in mice (n = 12) anesthetized with ketamine/xylazine at 7 T. Even without trigger-related delay, the peak latencies are in good agreement with the DIANA signals previously reported at 11.7T [3]. In summary**,** contrary to what Phi Van et al. reported, we successfully identified DIANA responses along the TC pathway in mice without trigger-related delay. DIANA fMRI is expected to play an important role in uncovering the brain's neural circuits with high spatiotemporal resolution.

References:

[1] Toi et al. *Science* 378, 160–168 (2022); [2] Phi Van et al., *Sci. Adv.* 10, eadl2034 (2024); [3] Keum et al. *bioRxiv*, doi: https://doi.org/10.1101/2024.07.31.606112