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Removal of stimulation artifacts in high-density Neuropixels recordings using sample clock-synchronized stimulation pulses

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Dear Editor,

Neuropixels (NP) probes [1,2] are high-density, integrated silicon electrodes that can simultaneously record from 384 neural channels across up to 5120 sites. These high-resolution recording devices have transformed systems neuroscience by enabling simultaneous large-scale recordings of hundreds of neurons. Initially popular for use in rodents and other small mammals (Neuropixels 1.0 and 2.0), Neuropixels are now also being used in larger non-human primates (Neuropixels 1.0 NHP) and even intraoperatively in humans. Interpreting data from these recordings relies heavily on precise spike sorting to accurately identify individual neurons. This process becomes even more challenging when neural recordings are performed concurrently with electrical stimulation, as stimulation artifacts can obscure the underlying neural signals.

Template estimation and subtraction is a widely used approach for removing stimulation artifacts from neural signals (see Refs. [3,4] for recent examples for multichannel arrays). However, in practice, variability in artifact shapes will arise when stimulation triggers are not precisely and consistently time-aligned with recording sample times (Fig. 1A; [5,6]). This misalignment can lead to substantial residual artifacts after template subtraction (Fig. 1B), limiting the effectiveness of the approach. Theoretically, when stimulation triggers are phase locked to the sample clock, the resulting artifacts should display very little variability, leading to smaller residuals (Fig. 1C). Notably, current Neuropixels probes do not offer a mechanism for hardware synchronization with the neural recording sample clock, further constraining artifact removal.

Accordingly, here, we present a novel method for improving stimulation artifact removal in Neuropixels recordings by leveraging the headstage's internal sample clock, to enable hardware-level synchronization of stimulation triggers to the sample clock. This synchronization is achieved by modifying the Neuropixels headstage and wiring its high-frequency 11.7MHz clock to a microcontroller, which then divides the clock down to 30KHz (Fig. 1D). The resulting 30KHz signal is precisely phase-locked to the 30KHz neural sample clock, thereby allowing synchronization of stimulation triggers to the neural data sample clock via a custom-built circuit (Fig. 1E and Supplementary Fig. 1; see Supplementary Methods and data availability statement for schematic and code). With this synchronization in place, stimulation artifacts should exhibit minimal variability in shape and amplitude (i.e., compare Fig. 1B and C, middle panels), allowing for reliable use of simple averaging to generate a precise, low-noise artifact template. This template can then be simply subtracted from the neural signal, resulting in near-perfect recovery of the underlying neural data, with negligible artifact residual or disturbance to the neural signals, and without requiring complex signal processing, filtering, modeling, or artifact estimation (Fig. 1C and B; compare right panels).

To validate our approach, we performed 384-channel recordings with Neuropixels 1.0 NHP probes in a rhesus macaque monkey. We recorded neural activity in the vestibular nuclei and surrounding areas in the brainstem during electrical stimulation of the vestibular nerve via vestibular prosthesis electrodes in the inner ear (Fig. 1D; see Ref. [7] for a recent review). Data recorded without stimulation trigger synchronization showed large artifact residuals after template subtraction (Fig. 1F), whereas data recorded with synchronization showed no discernible residuals, leaving action potentials clearly visible and undisturbed (Fig. 1G). The method worked well across all recording channels (examples across several channels shown in Supplementary Fig. 2). This is quantified in Supplementary Fig. 2C by measuring the total root mean square (RMS) voltage of each channel's waveform: approximately 200 μV before artifact removal, reduced to about 70 μV with unsynchronized artifact removal, and further reduced to ~20 μV with synchronized removal—essentially indistinguishable from recordings without stimulation. Additionally, our method remains robust in the presence of external noise sources (e.g., power supply interference), provided the amplifier does not saturate (Supplementary Fig. 3). To facilitate broader adoption, we also developed a software package (See data availability statement for github repository) that automatically removes artifacts and saves the processed data in the original SpikeGLX binary format, enabling seamless compatibility with common spike-sorting tools such as Kilosort.

Our approach leverages hardware-level synchronization to align electrical stimulation with the data acquisition system's internal clock, ensuring that each sampled artifact is precisely timed and highly

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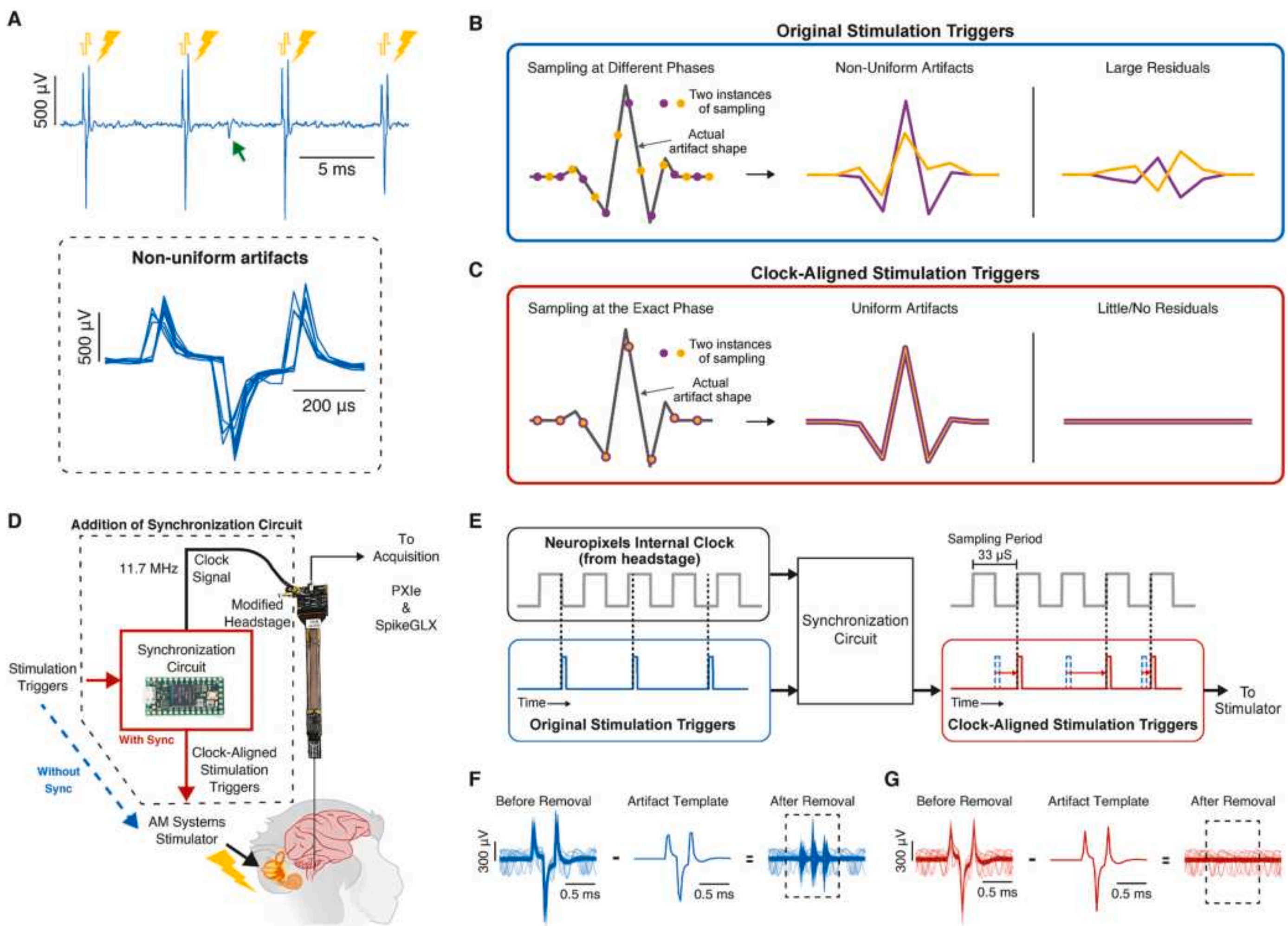

Fig. 1. Stimulation synchronization allows for exact sampling of stimulation artifacts. A) Top: An example neural recording showing stimulation artifacts (yellow symbols above) without synchronization, along with an action potential (green arrow), which is much smaller in amplitude. Bottom: These artifacts exhibit significant variability when aligned to unsynchronized stimulation triggers. B) Theoretical representation of two instances of the artifact being sampled when stimulation is not synchronized to the sample clock, resulting in large residuals after artifact removal. C) Similar to B but with stimulation synchronized to the sample clock, resulting in little to no residuals after artifact removal. D) Schematic of the synchronization circuit within the stimulation and recording systems. E) Schematic of how the synchronization circuit processes signals. The circuit takes in the original triggers and aligns them to the Neuropixels sample clock. F) Without stimulation synchronization, trigger-aligned artifact waveforms are non-uniform (left). Large residuals remain after template subtraction (right, dash-lined box). G) Similar to F but for the recording with stimulation synchronization. Note that the artifacts are highly uniform (left) and that little to no residuals remain after template subtraction (right, dash-lined box).

consistent across trials. This precise sampling of stimulation artifacts eliminates the need for complex algorithms to approximate or model artifact templates. Instead, with our hardware-based synchronization circuit, simple averaging and subtraction are remarkably effective for artifact removal. We note that, as with all artifact removal methods, our approach requires that stimulation artifacts must remain within a range that avoids driving the neural recording amplifiers into nonlinearity or saturation, conditions that would result in irreversible data loss and prevent recovery of the underlying signal through template subtraction. Additionally, the duration of the artifact template must be carefully constrained to ensure that it does not include evoked spiking that is reliably time-locked to the stimulation. As with other artifact removal techniques, our synchronization method also assumes that stimulation waveform parameters (e.g., amplitude, pulse shape) remain stable during the period used to create and subtract the template; however, a new template can be generated and used whenever stimulation parameters are modified. In addition, our method also requires that the experiment tolerate stimulation triggers aligned with the 30 kHz sample clock. This constraint is typically not limiting, as the stimulation interval resolution (33.33 μSec), is much shorter than the spike timing precision observed in most sensory systems —for instance, approximately 6 ms in the vestibular system [8]. Finally, the external stimulation device must be capable of accepting a hardware trigger timed to the actual stimulation pulses with sub-microsecond precision, in order to achieve consistent artifact waveforms.

In summary, we introduce a novel hardware-based strategy that aligns stimulation to the Neuropixels sample clock, thereby simplifying artifact removal in high-density neural recordings. This precise alignment ensures consistent sampling of artifact waveforms, enabling effective elimination. We validated this approach using *in vivo* recordings, which demonstrate reliable artifact removal with minimal residuals and no distortion of neural signals. Provided amplifier saturation is avoided, it remains robust even in the presence of external noise. While developed using Neuropixels 1.0 probes, synchronized template subtraction is broadly applicable to other high-density platforms, including Neuropixels 2.0, as long as the sample clock signals are accessible. However, the approach as presented does not synchronize multiple NP head-stages simultaneously since each head-stage generates its own free-running sample clock. In the case of NP 2.0, where each head-stage connects to two probes, the two probes within a given head-stage share the same sample clock and can therefore be synchronized to the stimulus simultaneously. Nevertheless, with further hardware development, beyond the scope of this letter, it may be possible to synchronize multiple head-stages to allow for more flexibility with multi-probe setup. More generally, because it leverages clock-level synchronization rather than platform-specific solutions or complex modeling, the method can be applied across a wide range of neuromodulation and neuroengineering systems that support hardware-level access to the sample clock.

CRediT authorship contribution statement

Kantapon Pum Wiboonsaksakul: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Investigation, Formal analysis, Data curation, Conceptualization. **Dale C. Roberts:** Writing – review & editing, Writing – original draft, Validation, Software, Methodology, Conceptualization. **Kathleen E. Cullen:** Writing – review & editing, Funding acquisition, Conceptualization.

Data availability statement

Details on Neuropixels head-stage modification, synchronizing circuit diagram and code, and the artifact removal codes with example data are available at https://github.com/CullenLab/NeuropixelsSYNC.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brs.2025.07.009.

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