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An assumption-free quantification of neural responses to electrical stimulations

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HIGHLIGHTS

- Assumption-free method for analyzing evoked responses.
- Robust identification of recording sites as responsive and non-responsive.
- Automatic categorization of responsive sites into different subtypes.
- Demonstrated on primate motor cortical responses to cerebellar stimulation.

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ABSTRACT

Background: Connectivity between brain regions provides the fundamental infrastructure for information processing. The standard way to characterize these interactions is to stimulate one site while recording the evoked response from a second site. The average stimulus-triggered response is usually compared to the pre-stimulus activity. This requires a set of prior assumptions regarding the amplitude and duration of the evoked response.
New method: We introduce an assumption-free method for detecting and clustering evoked responses. We used Independent Component Analysis to reduce the dimensions of the response vectors, and then clustered them according to a Gaussian mixture model. This enables both the detection and categorization of responsive sites into different subtypes.
Results: Our method is demonstrated on recordings obtained from the sensory-motor cortex of behaving primates in response to stimulation of the cerebello–thalamo–cortical tract. We detected and classified the evoked responses of local field potential (LFP) and local spiking activity (multiunit activity—MUA). We found a strong association between specific input (LFP) and output (MUA) patterns across cortical sites, further supporting the physiological relevance of the proposed method.
Comparison with existing methods: Our method detected the vast majority of sites found in the conventional, significant threshold-crossing method. However, we found a subgroup of sites with a robust response that were missed when using the conventional method.
Conclusion: Our method provides a useful, assumption-free tool for detecting and classifying neural evoked responses in a physiologically-relevant manner.

1. Introduction

Examining the connectivity between nearby or remotely located neurons is an important tool of studying neural processing in the central nervous system. One of the most commonly used methods to address the issue of connectivity between neuronal populations is by repeatedly stimulating one area (using electrical, magnetic, or optical drives), and recording the extracellular response triggered by this stimulation in another area (Cheney and Fetz, 1985; Jankowska et al., 1975; Shimazu et al., 2004; Yanai et al., 2007). This

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is an important tool for comprehending the nature and magnitude of the physiological connectivity between the stimulated and the recorded site. This approach also provides a critical test for verifying that recordings are indeed being made at a specific receiving site when no other physiological markers are available (Anderson and Turner, 1991).

To interpret the response to stimuli, the recorded signal is usually averaged around stimulus onset times, and compared to the baseline level of activity (e.g., Yanai et al., 2007). However, response patterns in many cases are complex (e.g., Edgley and Jankowska, 1987; Wilms et al., 2002; Zinger et al., 2013) and threshold crossing methods may fail to detect connected sites for various reasons. For example, responses that are comprised of both excitation and inhibition or responses with varying durations can be difficult to detect using threshold-crossing based methods. After detecting significant evoked responses, the response profiles are often sorted into different patterns in a qualitative manner, based on parameters determined by human observers (e.g., Magill et al., 2004). This approach frequently lacks the robustness required for an unbiased sorting process.

Here, we present a novel approach for detecting and sorting the evoked response of neural signals, which is practically assumption-free. The signal to be tested can be either the low-pass filtered local field potential (LFP, comprised mostly of synaptic activity) or the rectified high-pass filtered neural signal (the outcome of multiunit spiking activity, MUA). The first step involves computing the average normalized stimulus triggered average (nSTA) per site, similar to frequently used stimulus-triggered averaging (Rosenblith, 1959). Then, the dimension of the mean response is reduced in an unsupervised manner and finally, the obtained waveforms are clustered in a semi-automatic process. We show that this method can detect responsive sites without prior assumptions as to the specific shape or timing of the evoked response. We further show that this method can classify the obtained responses into physiologically meaningful subclasses.

Applying this method to cortical responses evoked by electrical stimulation in the superior cerebellar peduncle (SCP) revealed that different responsive cortical areas can be detected as well as classified based on their response characteristics. Moreover, the classification was consistent when using different neural signals (i.e., LFP vs. MUA), thus further confirming the physiological relevance of classifying response shapes as opposed to estimating response magnitude alone.

2. Methods

2.1. Behavioral task and electrophysiological recordings

Data were obtained from a *Macaca fascicularis* monkey. The monkey's care and surgical procedures complied with the Hebrew University Guidelines for the Use and Care of Laboratory Animals in Research, supervised by the Institutional Committee for Animal Care and Use. The monkey was trained to sit in a primate chair and perform a two-dimensional isometric wrist task, similar to previous experiments carried out in our lab (Yanai et al., 2007; Yanai et al., 2008).

After training, a recording chamber ($21 \times 21\ mm^2$) was attached to the monkey's skull above the hand-related motor cortex in a surgical procedure under general anesthesia. After a recovery and re-training period, extracellular recordings of motor cortical activity began. During recording sessions, glass coated tungsten electrodes (impedance 300–600 kΩ at 1000 Hz) were inserted through the chamber to different cortical sites, mostly in the primary motor cortex (M1). The signal obtained from each electrode was amplified ($\times 10^4$), and fed through two different online bandpass filters (300–6000 Hz for the single-unit data and 1–250 Hz for the LFP). The signal was then digitized at different sampling rates for the two signals (single unit: 25 kHz, LFP: 1 kHz).

Next, in a second operation, two low-impedance platinum-iridium electrodes (WeSense Ltd, Nazareth, Israel) were chronically implanted in the SCP ipsilaterally to the working hand. The trajectories were calculated to target the SCP based on a pre-operative MRI scan in which the SCP was identified stereotactically. The exact location of the electrode tips was verified in a second, postoperative MRI scan.

On each recording day, each electrode was inserted through the dura mater into the cortex, up to detection of a recording site where the signal was stable and neuronal activity was well-defined. A consecutive site was required to be at least 200 microns deeper than the previous one. At each site we used approximately 200 biphasic pulse (200 μs duration of each phase) stimuli, applied bipolarly between the two chronically implanted electrodes. Stimuli were delivered at 3 Hz, at an intensity of 150 μA, yielding traces with a maximal duration of 333 ms.

2.2. Data analysis

2.2.1. Computing the stimulus-triggered average of MUA

All analyses were implemented in Matlab (The MathWorks). The following process was applied to cortical data collected in each recording site separately. To extract the multiunit activity (MUA) from the full signal, we first removed the artifact caused by the SCP stimuli from the compound single-unit signal. This artifact led to a period of time during which either the amplifiers were saturated or the signal did not return to its pre-stimulus baseline level. To remove the artifact we first up-sampled the signal at eight times the original sampling rate (to 200 kHz), for a precise identification of the stimulus onset time. Next, we used existing methods for artifact removal (Hashimoto et al., 2002; Wichmann, 2000). Accordingly, we first used an iterative method to detect the peak amplitude of the artifact, thereby minimizing the jitter of onset time of each stimulation (assuming that the time to peak from electrical stimulation, which is not a physiological process, has very little variance). Next, we averaged the response to the electrical stimulation in a time window spanning up to 30 ms after stimulation, a period at which the response returned to its baseline level. Then, the averaged response was subtracted from each trace, and the period during which the signal was fully saturated (0.2–0.6 ms) was set to zero. Finally, the signal was down-sampled back to the original sampling rate. The resulting signal included only small residuals of the stimulus artifact (Fig. 1a and b).

Since the stimulus artifact varied between repeated sweeps (due to noise in the stimulus onset time and amplitude), some residuals of the stimulus artifact remained after this first stage. Therefore, in computing stimulus-triggered averages of the data (see below) we only considered data sections starting 1.8 ms after the stimuli.

After the artifact removal stage, the compound signal was high-pass filtered at 1 kHz (four pole Butterworth filter). This was done to make sure the signal did not contain residues of LFP in its bandwidth. The high pass filtered signal was then rectified, and smoothed by convolution with a 0.1 ms width Gaussian (Fig. 1c; Brosch et al., 1997; Legatt et al., 1979).

Next, for each recording site, the normalized stimulus-triggered average was calculated. First, the signal was truncated into traces spanning from −10 to +30 ms around each stimulus. Next, to remove possible outlier traces, each trace was averaged across time, yielding the mean activity level per trace. For each site, traces whose mean activity level was more than two medians of the absolute deviation (MADs) away from the median activity level for that site were eliminated from further analyses. This step did not have