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Cross-correlation of instantaneous amplitudes of field potential oscillations: A straightforward method to estimate the directionality and lag between brain areas

Avishek Adhikari [a], Torfi Sigurdsson [b], Mihir A. Topiwala [b], Joshua A. Gordon [b,c,*]

[a] *Department of Biological Sciences, Columbia University, New York, NY 10032, United States*
[b] *Department of Psychiatry, Columbia University, New York, NY 10032, United States*
[c] *Division of Integrative Neuroscience, New York State Psychiatric Institute, New York, NY 10032, United States*

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ABSTRACT

Researchers performing multi-site recordings are often interested in identifying the directionality of functional connectivity and estimating lags between sites. Current techniques for determining directionality require spike trains or involve multivariate autoregressive modeling. However, it is often difficult to sample large numbers of spikes from multiple areas simultaneously, and modeling can be sensitive to noise. A simple, model-independent method to estimate directionality and lag using local field potentials (LFPs) would be of general interest. Here we describe such a method using the cross-correlation of the instantaneous amplitudes of filtered LFPs. The method involves four steps. First, LFPs are band-pass filtered; second, the instantaneous amplitude of the filtered signals is calculated; third, these amplitudes are cross-correlated and the lag at which the cross-correlation peak occurs is determined; fourth, the distribution of lags obtained is tested to determine if it differs from zero. This method was applied to LFPs recorded from the ventral hippocampus and the medial prefrontal cortex in awake behaving mice. The results demonstrate that the hippocampus leads the mPFC, in good agreement with the time lag calculated from the phase locking of mPFC spikes to vHPC LFP oscillations in the same dataset. We also compare the amplitude cross-correlation method to partial directed coherence, a commonly used multivariate autoregressive model-dependent method, and find that the former is more robust to the effects of noise. These data suggest that the cross-correlation of instantaneous amplitude of filtered LFPs is a valid method to study the direction of flow of information across brain areas.

1. Introduction

Recent advances in multi-site recording technology have enabled researchers to sample local field potentials (LFPs) simultaneously from multiple brain regions (DeCoteau et al., 2007). A common interest in such studies is to determine whether one brain region is leading or lagging relative to another, and to estimate the time lag between putatively connected areas. Several groups have estimated the lag across brain areas using recordings of spike trains. Most of these studies estimate directionality by calculating the cross-correlation of spike trains of two areas (Alonso and Martinez, 1998; Holdefer et al., 2000; Lindsey et al., 1992; Snider et al., 1998). Other studies have used related approaches, such as calculating the cross-covariance of spike trains (Siapas et al., 2005), or different methods, such as the computation of spike-triggered joint histograms (Paz et al., 2009) or the change in phase-locking after shifting the spikes relative to the LFP (Siapas et al., 2005). Although such methods are effective, they are not applicable to studies that record only LFPs. This situation is common, as often spike trains cannot be sampled from multiple areas, or firing rates are too low to determine the directionality of functional connectivity across regions. Recording LFPs in areas with low firing rates can be advantageous, as LFPs can be sampled continuously, while spikes can occur infrequently and irregularly. LFP-based methods may therefore yield higher temporal resolution and greater statistical power than spike-based methods.

Existing methods such as Granger causality (Cadotte et al., 2010; Gregoriou et al., 2009; Popa et al., 2010) and partial directed coherence (PDC) (Astolfi et al., 2006; Baccala and Sameshima, 2001; Taxidis et al., 2010; Winterhalder et al., 2005) are able to estimate the directionality of functional connectivity using only LFPs. However, these methods are mathematically complex (Gourevitch et al., 2006), relying on multivariate models with a large number of free parameters, obscuring the intuitive understanding of what these

* Corresponding author at: Department of Psychiatry, Columbia University, 1051 Riverside Drive, Unit 87, Kolb Annex Room L174, New York, NY 10032, United States. Tel.: +1 212 543 6768; fax: +1 212 543 1174.
E-mail address: jg343@columbia.edu (J.A. Gordon).

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methods are actually computing. They can also be sensitive to noise (Taxidis et al., 2010; Winterhalder et al., 2005). Furthermore, such methods generally do not provide estimates of the time lag between brain areas.

Here we report a novel and mathematically straightforward method to estimate the lag between two brain areas that does not require spikes and that can be applied to datasets in which only LFPs have been acquired. The method requires that functional connectivity between the examined structures be accompanied by reasonably coherent activity within a specific frequency range. The method consists of determining the position (or "lag") of the peak of the cross-correlation of the amplitude envelopes of the LFPs after filtering for the frequency range of interest. Lastly, a non-parametric signed-rank test is performed to verify if the distribution of lags obtained from multiple experiments differs from zero.

To investigate its validity, this method was applied to a dataset in which both spikes and LFPs were recorded from the medial prefrontal cortex (mPFC), while only LFPs were sampled from the ventral hippocampus (vHPC). These areas were chosen because there is a unidirectional projection from the vHPC to the mPFC (Parent et al., 2009; Verwer et al., 1997), suggesting that activity in the vHPC should lead that in the mPFC. Moreover, we have shown theta-frequency (4–12 Hz) coherence between these structures during behavior (Adhikari et al., 2010), suggesting that directionality analysis can be performed in this frequency range with the amplitude cross-correlation measure.

Using the amplitude cross-correlation method, we demonstrate that the vHPC leads the mPFC in the theta-frequency range, with a lag consistent with estimates of the conduction delay of this pathway. Furthermore, there is good agreement between vHPC–mPFC lags calculated with this method and those calculated from phase locking of mPFC spikes to vHPC theta oscillations. Finally, a consistent lag between the vHPC and the mPFC was only found in the theta, but not in the delta and gamma-frequency ranges, in line with studies suggesting that theta-frequency oscillations drive functional connectivity between the hippocampus and the mPFC (Adhikari et al., 2010; Jones and Wilson, 2005; Siapas et al., 2005). The current method was also compared to partial directed coherence (PDC), an existing method to calculate the directionality of functional connectivity with LFPs. This method, similarly to the amplitude cross-correlation method, also demonstrated that the vHPC leads the mPFC in the theta-range. To further compare the two methods, both were applied to simulations in which pink noise was added to biological signals. Strikingly, PDC was more susceptible to errors induced by noise than the amplitude cross-correlation method. These results show that the cross-correlation of the amplitude of filtered field potentials may provide a valid, relatively robust estimation of the lag and the directionality of information flow across brain areas.

2. Materials and methods

2.1. Animals

Three to six month old male wildtype 129Sv/Ev mice were obtained from Taconic (Germantown, NY, USA). Seventeen mice were used for the simultaneous mPFC and vHPC recordings. Sixteen mice were used for the simultaneous vHPC and dorsal hippocampus (dHPC) recordings. An additional cohort of five C57/Bl6 mice bred at Columbia University was used for the simultaneous dHPC and mPFC recordings, from which mPFC single units were isolated. The procedures described here were conducted in accordance with National Institutes of Health regulations and approved by the Columbia University and New York State Psychiatric Institute Institutional Animal Care and Use Committees.

2.2. Surgery and microdrive construction

Custom microdrives were constructed using interface boards (EIB-16, Neuralynx, Bozeman MT) fastened to a Teflon platform, as described previously (Adhikari et al., 2010). Briefly, animals were anesthetized with ketamine and xylazine (165 and 5.5 mg/kg, in saline) and secured in a stereotactic apparatus (Kopf Instruments, Tujunga, CA). Screws were implanted on the posterior and anterior portions of the skull to serve as ground and reference, respectively. mPFC electrodes were implanted in the deep layers (V/VI) of the prelimbic cortex, at +1.65 mm anterior, 0.5 mm lateral and 1.5 mm depth, relative to bregma. vHPC electrodes were implanted in the CA1 region at 3.16 mm posterior, 3.0 mm lateral and 4.2 mm depth, and dHPC electrodes were targeted to 1.94 posterior, 1.5 lateral and 1.3 mm depth. Depth was measured relative to brain surface.

2.3. Behavioral protocol

Animals were permitted to recover for at least one week or until regaining pre-surgery body weight. Mice were then exposed to a small rectangular box in the dark, in which they foraged for pellets for 10 min for the mPFC–vHPC and vHPC–dHPC datasets. Mice performed an alternation task in a T-shaped maze for the dHPC–mPFC dataset as described in (Sigurdsson et al., 2010).

2.4. Data acquisition

Recordings were obtained via a unitary gain head-stage preamplifier (HS-16; Neuralynx) attached to a fine wire cable suspended on a pulley so as not to add any weight to the animal's head. LFPs were recorded against a reference screw located at the anterior portion of the skull. Field potential signals were amplified, bandpass filtered (1–1000 Hz) and acquired at 1893 Hz. Multiunit activity from the mPFC was recorded simultaneously from the same electrodes used to obtain LFPs; multiunit signals were bandpass filtered (600–6000 Hz) and recorded at 32 kHz. Events exceeding a threshold of 40 μV were selected for analysis of phase-locking to theta (see below). Both LFP and multiunit data were acquired by a Lynx 8 programmable amplifier (Neuralynx) on a personal computer running Cheetah data acquisition software (Neuralynx). The animal's position was obtained by overhead video tracking (30 Hz) of two light-emitting diodes affixed to the head stage.

2.5. Cross-correlation analysis

2.5.1. Band-pass filtering

Data was imported into Matlab for analysis using custom-written software. To calculate the lag between the vHPC and the mPFC, signals were initially band-pass filtered between 7 and 12 Hz. A finite impulse response filter of order n, where n is the sampling frequency, was implemented with a Hamming window, utilizing the MATLAB function *fir1*.

2.5.2. Instantaneous amplitude using the Hilbert transform

The Hilbert transform of each signal was computed with the MATLAB function *hilbert*. The output of the Hilbert transform is a vector containing complex numbers that has the same number of elements as the input signal. The real portion of the complex number is the input itself, while the imaginary part is the input LFP shifted by 90° ($\pi/2$ radians). The absolute magnitude of the complex number at a given time point is the power of the filtered signal at that sample. The magnitude of a complex number is the length of the vector in the complex plane.