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Untangling cross-frequency coupling in neuroscience

Juhan Aru[1,2], Jaan Aru[1,3,4,5,10], Viola Priesemann[1,3,6], Michael Wibral[7], Luiz Lana[1,3,4,8], Gordon Pipa[9], Wolf Singer[1,3,4] and Raul Vicente[1,3,4,7,10]

Cross-frequency coupling (CFC) has been proposed to coordinate neural dynamics across spatial and temporal scales. Despite its potential relevance for understanding healthy and pathological brain function, the standard CFC analysis and physiological interpretation come with fundamental problems. For example, apparent CFC can appear because of spectral correlations due to common non-stationarities that may arise in the total absence of interactions between neural frequency components. To provide a road map towards an improved mechanistic understanding of CFC, we organize the available and potential novel statistical/modeling approaches according to their biophysical interpretability. While we do not provide solutions for all the problems described, we provide a list of practical recommendations to avoid common errors and to enhance the interpretability of CFC analysis.

Addresses
[1] Max-Planck Institute for Brain Research, Frankfurt am Main 60528, Germany
[2] École Normale Supérieure de Lyon (UMPA), Lyon 69364, France
[3] Frankfurt Institute for Advanced Studies, Frankfurt am Main 60438, Germany
[4] Ernst Strüngmann Institute, Frankfurt am Main 60528, Germany
[5] Institute of Public Law, Tartu University, Tallinn 10119, Estonia
[6] Max-Planck Institute for Dynamics and Self-Organization, Göttingen 37077, Germany
[7] MEG Unit, Brain Imaging Center, Goethe University, Frankfurt am Main 60528, Germany
[8] Brain Institute, Federal University of Rio Grande do Norte, Natal 59056, Brazil
[9] Institute of Cognitive Science, University of Osnabrueck, 49069, Germany
[10] Institute of Computer Science, University of Tartu, Tartu 50409, Estonia

Corresponding author: Vicente, Raul (raulvicente@gmail.com)

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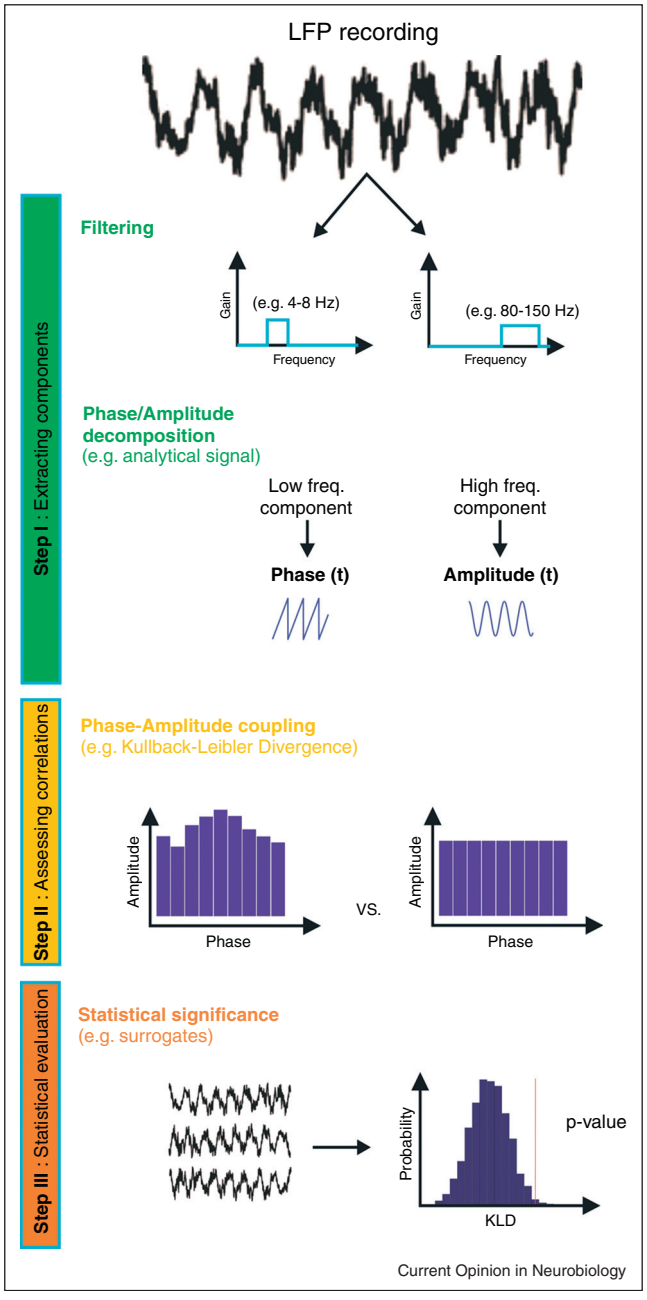
Cross-frequency coupling (CFC): how much is that in real money?

One of the central questions in neuroscience is how neural activity is coordinated across different spatial and temporal scales. An elegant solution to this problem could be that the activity of local neural populations is modulated according to the global neuronal dynamics. As larger populations oscillate and synchronize at lower frequencies and smaller ensembles are active at higher frequencies [1], CFC would facilitate flexible coordination of neural activity simultaneously in time and space. In line with this proposal, many studies have reported such cross-frequency relationships [2–4]. Especially phase–amplitude CFC, where the phase of the low frequency component modulates the amplitude of the high frequency activity, has been claimed to play important functional roles in neural information processing and cognition, for example, in learning and memory [4–7,8••]. Furthermore, changes in CFC patterns have been linked to certain neurological and mental disorders such as Parkinson's disease [9–10,11•], schizophrenia [12–14] and for example social anxiety disorder [15]. Therefore, CFC is potentially essential for normal brain function and understanding of CFC patterns can be crucial for diagnosing and eventually treating various disorders.

The classical analysis of CFC seems very straightforward (Figure 1) and is widely used. However, not all signatures of CFC as detected by this analysis method need to be due to interactions between different physiological processes occurring at different frequencies, as is commonly reported. It has been previously shown that signals with abrupt changes lead to spurious CFC results [16] (see *Supplementary results: Examples of spurious CFC* for a related example). The roots of this problem are much more general. Let us take as an example the Van der Pol oscillator, which is a very simple non-linear relaxation oscillator. Conducting the CFC analysis on this oscillator would indicate that the phase of the low frequency components modulates the activity of the higher frequencies. However, despite strong CFC signal there is no simple physical interpretation for the different frequency components of the oscillator, and even less for their interaction. Indeed, any interpretation in terms of modulating or causally interacting frequencies is misleading as the spectral correlations are related to the non-linear characteristics of a single oscillator (see *Supplementary results: Examples of spurious CFC* for a thorough description of this example).

Figure 1



Typical approach to analyze phase–amplitude cross-frequency coupling. Step 1: Extracting the relevant components. This step is implemented by band-pass filtering and extraction of phase and amplitude dynamics for the relevant frequency bands. Step 2: Assessing correlations between components. This stage requires the computations of appropriate correlation or dependency measures between amplitude and phase. Therefore, a general measure of phase–amplitude coupling is to precisely quantify how much the histogram of mean amplitude versus phase deviates from a uniform distribution. Step 3: Statistical evaluation. Parametric or non-parametric approaches comparing to suitable surrogate data can be used to assign a *P*-value to the observed coupling strength.

This hints that the current analysis of CFC is inherently ambiguous regarding the nature and origin of the observed correlations between the frequency components. A significant CFC measure can be observed in case there are true modulations between subsystems oscillating at different frequencies. However, it can be also observed under very generic conditions that imply no coupling. Similarly to the example above, any non-linear response where fast components are short-lived compared to the slow components of the signal would produce a significant CFC. In particular this means that current CFC measures of phase-to-amplitude coupling are not specific enough for one to automatically conclude, as it is almost invariably done in the literature, that the phase of a low frequency oscillation modulates the power of high-frequency activity. The same holds for CFC measures of amplitude-to-amplitude, or phase-to-phase coupling.

We wish to emphasize that we do not question the possible importance of CFC as a phenomenon. In fact, we believe that such a mechanism would be an elegant solution to several computational demands the brain has to cope with [1]. However, precisely because of the potential relevance of CFC for understanding the healthy and the pathological brain it is necessary to be aware of the pitfalls and misinterpretations in the methodology currently applied. We hope that the careful assessment of concerns will eventually strengthen the power of CFC analysis as an experimental tool.

The outline of the rest of the paper is as follows: First, we shall point out fundamental caveats and confounds in the current methodology of assessing CFC. Some of these points are original and some others have been known in other fields for years, yet all share the characteristic of being unattended in many of the current studies. Our literature review of the phase–amplitude CFC studies from the years 2010–2014 shows that these issues are relevant and timely (see *literature review* in the *Supplementary material*). Second, we propose an organization of different approaches to CFC analysis according to their biophysical interpretability and statistical inference approach. Finally, we outline some practical recommendations for CFC analysis.

In this Opinion article we cannot offer solutions for all the problems of CFC analysis and interpretation that are described, but our hope is that alerting the community to these problems will eventually lead to novel solutions.

Caveats and confounds of the CFC analysis

In this section we concentrate on what we call the classical CFC analysis — it is illustrated and explained in Figure 1. Any result of this analysis can be used to classify different conditions but only as a marker that is devoid of concrete and clear physiological interpretation. To give a physiological interpretation to CFC, one needs to know the set

of potential mechanisms responsible for neural coupling. This set of mechanisms is only beginning to emerge (discussed below). We now discuss some main methodological confounds that make it difficult to build connections between the CFC measure and the underlying neurophysiological processes. More caveats and confounds and examples of spurious CFC can be found in the *Supplementary results*.

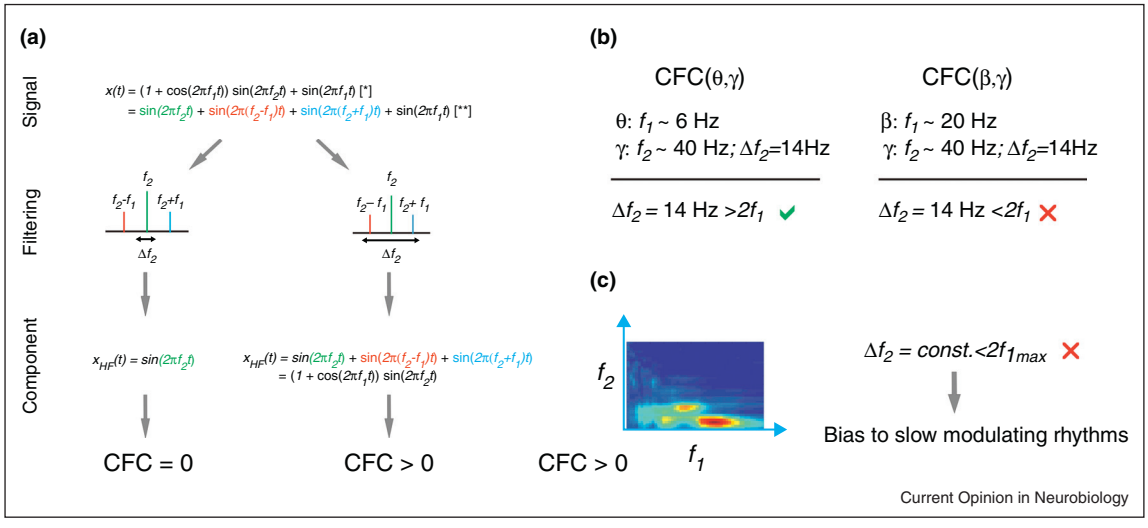
Instantaneous phase and amplitude: when are they meaningful and when not?

Standard phase–amplitude CFC analysis proceeds by first selecting two frequency bands followed by the computation of some index for the correlation or dependency between the phase of one band and the amplitude of the other (Figure 1). The phase and amplitude values extracted from filtered signals can unfortunately only be interpreted in a meaningful way, that is, as representing physiological oscillations, if a number of basic requirements are met. The same holds for CFC analyses based on them. In *Supplementary discussion: Conditions for a meaningful phase* we present a short but rather thorough review of the conditions that must be met for a meaningful interpretation of phase and amplitude values. The main conclusion is — not that surprisingly — that a clear peak in the power spectrum of the low frequency component is a prerequisite for a meaningful interpretation of any CFC pattern. Our literature review shows that even these well-known conditions were and are not always met in the literature, resulting in a strong over-interpretation of phase and amplitude (see *literature review* in the *Supplementary material*).

The importance of the bandwidth

The two components entering a phase–amplitude CFC analysis after filtering the signal, are determined by the center frequencies and bandwidths of the filters used to isolate them. Our literature review shows that majority of studies proceed by scanning the center frequencies for the phase and amplitude components while keeping a fixed bandwidth of a few Hz. However, this choice of bandwidth is important because it defines what is considered as a component and how the component's power or group phase changes in time (Figure 2). Thus, it is not the same thing to scan a center frequency from 20 Hz to 60 Hz with a bandwidth of 2 Hz or to consider at once the band centered at 40 Hz with a width of 21 Hz — different effects will be observed. Unfortunately, little or no justification is given to the choice of parameters in most analyses. The choice of bandwidth for the phase component is constrained by the condition of having a meaningful phase and is therefore often correctly chosen to be narrow. However, one also needs to be careful with the bandwidth size for amplitude — if the bandwidth of the higher frequency component (f2) does not include the side peaks produced by the lower frequency (f1), then CFC cannot be detected even if it is present (Figure 2a). Thus certain parameter values usually chosen in the literature can bias the CFC measures towards obtaining

Figure 2



Mathematical decomposition and filtering bandwidth are key parameters to infer and interpret the presence of CFC. **(a)** (Signal) The very same signal can be decomposed into different mathematically equivalent representations ([*] or [**]). The choice of the representation leads to different interpretations regarding the interactions of the components (Filtering and Component). Different filtering bandwidths around the same frequency can lead to different results depending on whether the bandwidth includes modulating sidebands or not. **(b)** For a fixed bandwidth of the modulated frequency, only a range of modulating frequencies can be captured. For example, in the simplified harmonic case, a bandwidth of 14 Hz around a frequency of 40 Hz would allow detection of a potential modulation from a 6 Hz rhythm, but not from a 20 Hz oscillation. **(c)** When scanning the modulating (f_1) and modulated (f_2) frequencies, a fixed bandwidth biases CFC analysis and favors low frequencies for f_1.

false negative results (see *Supplementary Discussion: The importance of the bandwidth*). See also [17].

Non-stationarity and spectral correlations: two sides of the same coin

Most neuronal signals that we measure are non-stationary. Time-varying sensory stimuli, top-down influences, neuromodulation, endogenous regulatory processes and changes in global physiological states render neuronal dynamics non-stationary. In contrast to a stationary process, a non-stationary process in general exhibits spectral correlations between components of its Fourier expansion [18]. These correlations may be misinterpreted as CFC. The underlying reason for these spectral correlations is that in constructing the spectrum, we decompose a process which is by definition not time-invariant (non-stationary) into the eigenvectors of the time-shift operator, that is, the complex exponentials in the Fourier expansion. Therefore, in the non-stationary case, there are two possible scenarios leading to positive CFC measures:

One scenario is that physiological processes indeed interact. This interaction then leads to non-stationarities, and at the same time we observe spectral correlations in the Fourier representation. For example, if the phase of a neural input oscillating at theta frequency modulates the amplitude of local gamma oscillations, both obtained from the same LFP recording, the statistical properties of the gamma oscillation amplitude series will change in time, as does theta phase. Specifically, their properties will vary in time only to be repeated after a full cycle of the slow oscillation, and thus exhibiting a particular type of non-stationarity called cyclo-stationarity [19].

The other and problematic scenario is that *unspecific* non-stationarities (that is, *any kind of* change of the statistical properties of the signal), not related to or caused by coupling of neural processes, will also be reflected in spectral correlations which could be over-interpreted as the result of causal interactions among frequency specific neuronal processes. This second scenario can occur if non-stationary input to a given area simultaneously affects the phase of a low frequency component and increases high-frequency activity (common drive to different frequency components of the same signal). For example, typical evoked potentials affect a broad range of frequency components [20]. In this case, high-frequency amplitude increases occur preferentially for certain phases of slow oscillations even without any need of interaction between the two rhythms.

Hence, non-stationary input to a given area can generate correlations between bands, which are not necessarily a signature of interactions between these bands. The argument goes well beyond the relationship between sensory stimulation and CFC in sensory areas: if a brain area under a recording electrode receives time-varying input from any other brain area, this input might generate similar dependencies across frequency components (Figure 3a). The problem is that usually one has no control over the timing of the internal input to the examined brain area (Figure 3b). If this internal input leads to an increase of phase locking for lower frequencies (Figure 3c, left) and at the same time elicits an increase in power at higher frequencies (Figure 3c, middle) phase–amplitude coupling will be observed (Figure 3c, right). The combination of increased activity at high-frequencies and phase-locking to the stimulus of lower frequencies is sufficient to obtain significant measures in standard CFC analysis. Thus, phase–amplitude coupling measured anywhere in the brain can be potentially explained by common influence on the phase and amplitude, without the phase of a low frequency oscillation modulating the power of high frequency activity. In the supplementary materials we illustrate this scenario with examples from the electroretinogram and LFP recorded in the optic tectum of a turtle (Pseudemys scripta elegans) and human intracranial recordings (Supplementary Figures 3 and 4).

Therefore, the key issue is to distinguish whether the observed phase–amplitude correlation between two bands is due to common drive, generated by external or internal input or whether the correlation is due to a causal interaction between rhythms (which, of course, could also be triggered by the input). Recently, a new approach [21] has been developed to measure transient phase–amplitude coupling directly in an event-related manner. Whereas ideally, their approach of analyzing phase–amplitude relations with respect to the stimulus onset should avoid some event-related artifacts, it is questionable whether the marker actually works as intended (see *Supplementary results: Phase–amplitude coupling for event-related potentials*). Ultimately solving these questions requires a formal causal analysis between the spectral variables of different bands (see also *Supplementary discussion: Causality methods*).

Analysis of between-channel phase–amplitude coupling [22,23•] is less likely to be the result of a driving input to a single area. In this research intracranial human data was used to identify the spatial maps of the low frequency (phase providing) and high frequency (amplitude providing) components. From the size and other characteristics of these maps one can conclude that the low-frequency and high-frequency components are separable in the brain. This result is important as such between-channel CFC cannot be created by non-stationary input to one area. However, these findings do not fully solve the underlying problem as these different generators could still be 'coupled' by a common driver influencing both generators, rather than by a direct interaction between them.

Figure 3

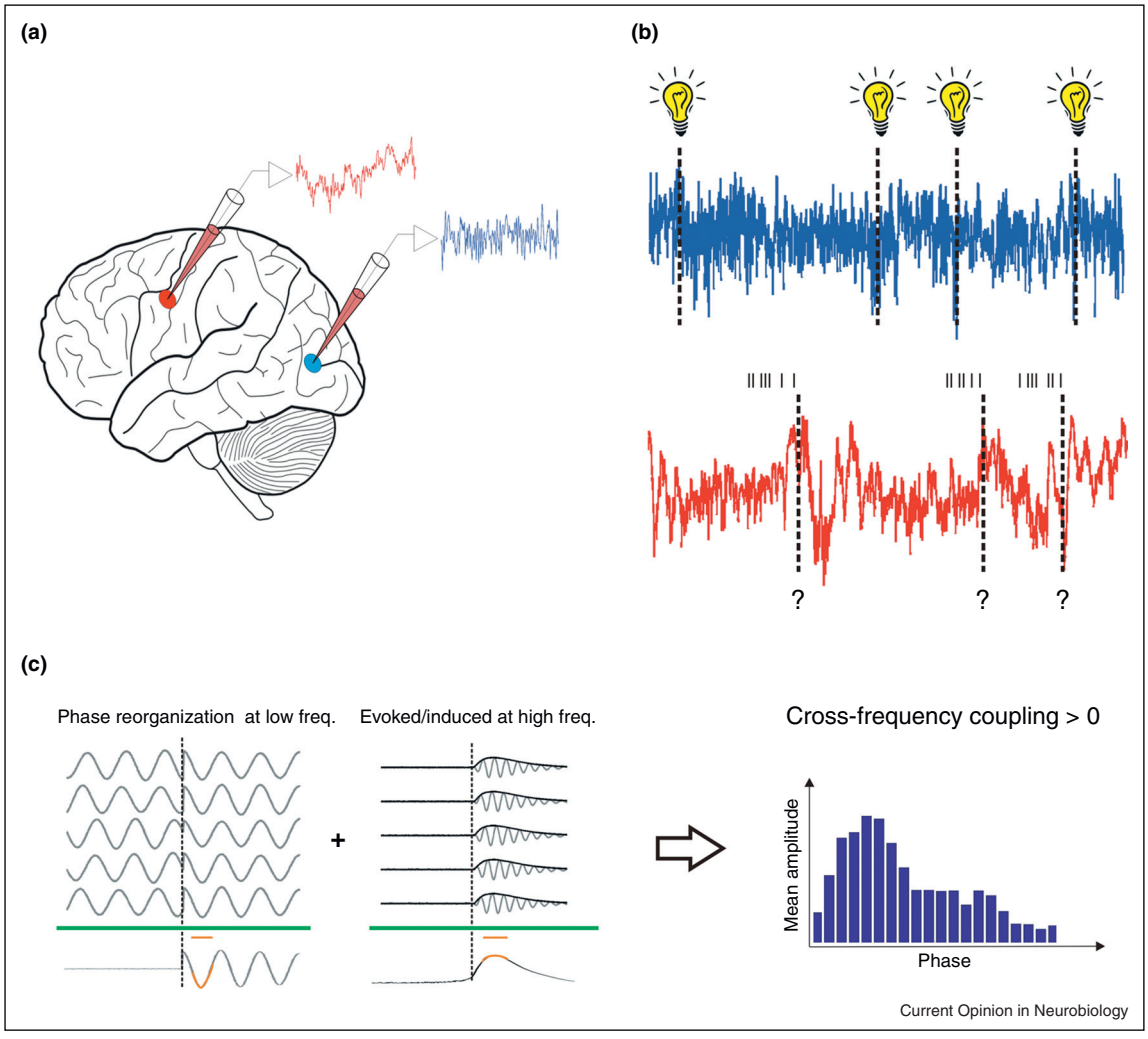


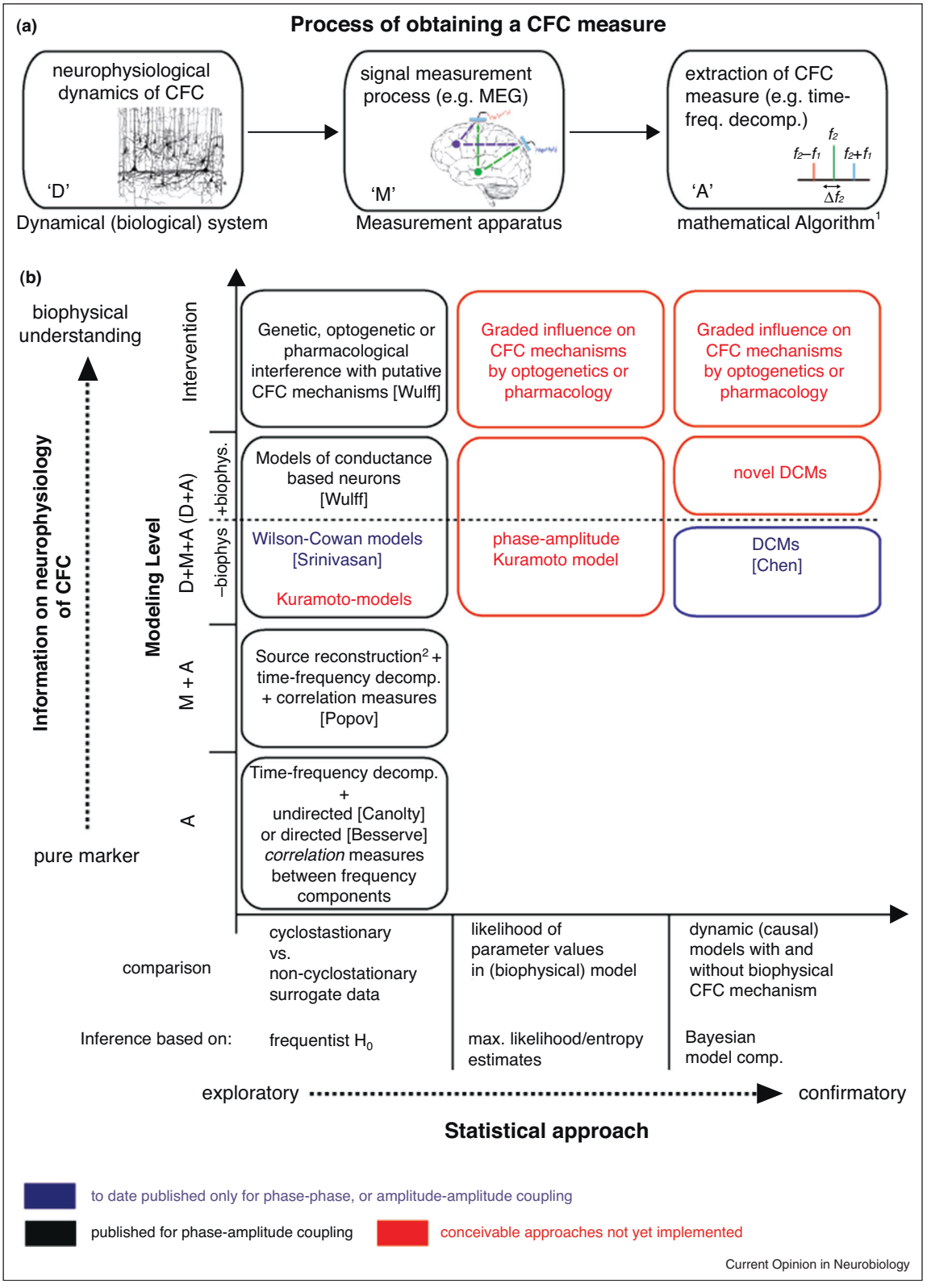
Illustration of how time-varying input can lead to false-positive CFC. **(a)** Illustration of recordings in a cortical sensory area (blue) and a higher cortical area (red). **(b)** Importantly, both can be subject to non-stationary neuronal input but its timing can only be determined for the sensory area. **(c)** Knowing the input timing is necessary to perform phase locking analysis, distinguish between evoked/induced responses and to disambiguate the origin of CFC. Without this additional information the results of the CFC analysis remain ambiguous.

In conclusion, the above considerations imply that the current phase–amplitude CFC measure is constitutive for the non-stationary responses of driven systems and therefore is not a very specific marker of biophysical coupling. From a mathematical perspective the key aspect is that any consistent response to input, whatever its shape, implies a certain phase locking between its different Fourier components [24,25]. Thus, if the power of any of the fast components lasts a bit more or a bit less than the period of a slow component, then its amplitude will accumulate preferably at certain phases of the slow component. This is all that is needed to give rise to phase–amplitude CFC, as measured for example by the modulation index [26]. It is therefore necessary to recognize that beyond the phase–amplitude CFC index, which is just an index at the signal level, additional information about how the process under study reacts to input and the statistics of the input itself are needed to better resolve the origin of such correlations. Analyses of surrogate data can help to remove some of the ambiguity but only offers partial remedies to the problem, as we shall discuss next.

Surrogate data: none are perfect but some are better than others

After some index of CFC has been estimated one needs to rely on statistical inference to reach a conclusion about the statistical significance of the measure. Currently, most studies of CFC rely on the frequentist approach of using surrogate data to estimate a *P*-value. Some issues related to generation of the surrogate data are discussed here. For the state of some Bayesian approaches see Figure 4 in the

Figure 4



Organization of approaches to CFC. **(a)** The process by which a researcher obtains a CFC measure. 'D' signifies the biological dynamical system (that may or may not have biological CFC). 'M' signifies the physical transduction and measurement process, including all physical (unavoidable) filtering distortion, instrument noise and potentially mixing processes. The measurement process can generate CFC in absence of biological CFC. 'A' signifies

section '*Organization of modeling/statistical approaches to CFC*'.

A suitable surrogate construction should only destroy the specific cyclo-stationarities related to the hypothesized CFC effect, while keeping all the unspecific non-stationarities and non-linearities of the original data. Often, it is impossible to construct perfect surrogates that selectively destroy the effect of interest but some approaches are more conservative than others. In our context a sensible requirement is to construct surrogates that minimize the distortion of both phase and amplitude dynamics for each frequency component. If data are organized in detectable repetitive events such as trials locked to an external stimulus or to saccades, shuffling the full/intact phase or amplitude components between different events seems the most straightforward approach. Unfortunately, the very existence of an event-related potential implies that some frequency components are both locked to the event and between themselves. Consequently, this strategy to obtain surrogates alone cannot discern the source of modulation. Future developments of methods to partial out the common drive effect of the event could help to test the significance of a direct phase–amplitude modulation.

Finding appropriate surrogate data for a single continuous stream of data comes with its own challenges. For example, phase scrambling does not meet the minimal distortion criterion, as the generated surrogate data are fully stationary after scrambling, that is, the non-stationarities of interest and the unspecific non-stationarities are both destroyed alike [27]. A significantly larger CFC in the original data may in this case be due to the removal of non-stationarities not specifically related to physiological CFC. Another approach has been using block re-sampling [3] where one of the continuous time series (i.e. the instantaneous phase) is simultaneously cut at several points and the resulting blocks permuted randomly. This method suffers again from destroying in excess the non-stationary structure of the original series. More conservative surrogates can be obtained by minimizing the number of blocks by cutting at single point at a random location and exchanging the two resulting time courses [3]. Repeating this procedure leads to a set of surrogates with a minimal distortion of the original phase dynamics.

Thus, while perfect surrogate data that selectively disrupt phase–amplitude coupling might be impossible to build (as is the case for most types of non-linear interactions) conservative approaches that minimize distortion of phase and amplitude dynamics can reduce the number of false positives.

CFC modulation across conditions

Several studies have reported significant changes in phase–amplitude CFC with variations of experimental parameters or across two different conditions. The modulation of CFC by the task or experimental condition has then been taken as an indication of its physiological role [4,5,7,28]. However, for now there is only little reason to believe that this modulation could not be due to side-effects of more basic changes between the conditions.

Since the power of bands directly influences the range within which they can modulate or be modulated, it is possible that changes in CFC correlations are a direct consequence of changes in power spectra. For example, changes in the observed CFC can have their origins in the fact that power changes affect the signal-to-noise ratio of phase and amplitude variables and their correlations (e.g. [29]). It is thus necessary to control whether correlations between CFC and other behavioral or physiological variables might be simply due to changes in, for example, the strength or frequency of oscillations. Unfortunately, our

(Figure 4 Legend Continued) the mathematical algorithm applied to the measured data to obtain a figure of merit for CFC. Typically this step involves filtering or a time–frequency decomposition, and a linear or nonlinear correlation measure. As shown in the main text, also this process can give rise to CFC in absence of biological coupling, for example, by ignoring the limits of time–frequency analysis in the face of non-stationarities. **(b)** Two-dimensional organization of CFC approaches. The *x*-axis sorts the approaches by the statistical inference technique that is used. Frequentist H_0 based approaches just test for presence of absence of CFC in the measured data, while maximum likelihood or Bayesian approaches perform inference on coupling parameters in models — and hence only appear for dynamic models with coupling parameters. The *y*-axis indicates the part(s) of the process in (a) that are modeled: 'A' — just extracting a CFC measure from measured data is equivalent to modeling this extraction process itself. 'M + A' — the generative model now comprises the measurement process and the measure extraction. 'D + M + A' — the modeling comprises a dynamic model of the biological process, either via a dynamic proxy process that models only the bare essentials of the dynamics (like a Kuramoto model for phase–phase coupling), or has biological detail (like a Hodgkin–Huxley model with some explicit mechanisms implementing CFC). Approaches that were only published for phase–phase or amplitude–amplitude coupling are highlighted in blue text, conceivable approaches, that have to our knowledge not been implemented at all yet are highlighted in red. (1) The fact that we use some numbers (measured data) and feed them to a mathematical algorithm to obtain other numbers (the CFC measure) can be modeled by just executing the algorithm. Nevertheless it is a part of the CFC process model. (2) By source reconstruction we mean any inversion of the measurement process, for example, unmixing via ICA, electromagnetic source reconstruction in electro-encephalography or magnetoencephalography, or removal of measurement noise. We provided some representative references on the figure: Besserve, M., Scholkopf, B., Logothetis, N.K. & Panzeri, S. Causal relationships between frequency bands of extracellular signals in visual cortex revealed by an information theoretic analysis. *Journal of Computational Neuroscience* **29**, 547–566 (2010). Canolty, R.T., *et al.* High gamma power is phase-locked to theta oscillations in human neocortex. *Science* **313**, 1626–1628 (2006). Chen, C.C., *et al.* A dynamic causal model for evoked and induced responses. *Neuroimage* **59**, 340–348 (2012). Popov, T., Steffen, A., Weisz, N., Miller, G.A. & Rockstroh, B. Cross-frequency dynamics of neuromagnetic oscillatory activity: two mechanisms of emotion regulation. *Psychophysiology* **49**, 1545–1557 (2012). Srinivasan, R. Thorpe, S. & Nunez, P.L. Top-down influences on local networks: basic theory with experimental implications. *Front Comp Neuroscience* **7**, 1:15 (2013). Wulff, P., *et al.* Hippocampal theta rhythm and its coupling with gamma oscillations require fast inhibition onto parvalbumin-positive interneurons. *Proc Natl Acad Sci U S A* **106**, 3561–3566 (2009).

literature review shows that in around half of the reviewed studies where conditions are compared, changes in the power spectrum across the conditions are not considered. If the data permit, it is therefore highly recommended to rely on stratification techniques (e.g. [30]) to obtain a subset of matched trials in which the distribution of power across trials is identical for both the phase and amplitude frequency bands in the two conditions to be compared.

In general, as CFC is a statistic based on the correlation of certain variables, it is necessary to control for the explanatory power of these variables themselves, before a specific role for the correlation might be distilled.

Organization of modeling/statistical approaches to CFC

Until now we have focused on what we call the classical approach (see Figure 1) to assess phase–amplitude CFC effects consisting of: first isolating frequency components, second assessing their dependencies, and third computing *P*-values based on surrogates. We have described how difficult it is at this stage to draw any conclusions about the biophysical mechanisms underlying these measures. However, different frameworks exist to assess relationships among rhythmic processes from experimental time series. A short description of some frameworks can be found in the *Supplementary discussion*. For the purpose of understanding the role of different frameworks in gaining physiological understanding of CFC, we have found it useful to organize them according to their biophysical interpretability and statistical inference approach (Figure 4).

We believe the location of the method along those axes (Figure 4) has to be taken into account to avoid over-interpretations of the results of a CFC analysis. The first section of this paper can be in fact seen as an explanation as to why some models including the classical approaches are positioned very close to the 'marker' section. For example, a simple correlation-based quantifier as provided by classical approaches is probably all that is needed, if the sole purpose of CFC analysis is to have a marker to classify different conditions (e.g. disease states). If one insists that this marker should be more specific than say just changes in the power-spectrum, already more work is needed, as discussed above. Finally, if the aim is to attach a well-defined physiological meaning to the observed CFC pattern, it is imperative to have either a generative model or additional external information, such as obtained from a direct perturbation of the putative physiological CFC mechanism, to link signal and underlying processes. Both of these latter approaches require a biophysical theory to be put forward as to how a neuron or an ensemble of neurons physically implements the coupling. For the moment, potential biological mechanisms of CFC are only starting to be discovered.

Indeed, whilst there is extensive knowledge about the physiological mechanisms responsible for different frequency components [1], not much is known about the cellular and network mechanisms of the *interactions* between these components [4]. Only recently some evidence about concrete mechanisms of interaction has been obtained from intervention studies in physiological systems and computational models. For example, by using transgenic mice it has been shown on the level of LFPs that hippocampal theta–gamma coupling depends on fast synaptic inhibition [31] and NMDA receptor-mediated excitation of parvalbumin-positive interneurons [32]. CFC at the LFP level has also been observed between alpha oscillations at the infragranular layer and gamma activity at the supragranular layers [33••]. Recently, it was also shown that feedback inhibition enables CFC at the level of membrane potential fluctuations [34••]. Thus, biological mechanisms for CFC can occur at the population level, at the single neuron level, or both. The most parsimonious explanation to account for these findings is that the low frequency oscillation reflects periodic fluctuations of the membrane potential and thus excitability, which in turn gate the occurrence of higher frequency activity in a phase specific manner [34••]. Typically this higher frequency activity reflects spikes which could display a rhythmic pattern (possibly reflected as gamma oscillations).

Along with plausible biological mechanisms, one also needs to take into account possible alternative explanations for non-zero CFC measures. In this perspective we have tried to aid the interpretation of CFC by demonstrating that although CFC patterns are typically interpreted as reflecting physiological coupling, they can be also generated by biological processes unrelated to direct coupling between different neural processes, and by methodological pitfalls. In the following section we compiled some practical recommendations to help to avoid some of these errors. Taking these alternative explanations into account and controlling for them experimentally will help towards a clear interpretation of CFC results.

As laid out above, interventions would be ideal to study CFC. When an intervention is not possible, another principled approach to test for the presence of biophysical CFC is a formal comparison of computational models that do or do not incorporate biophysical CFC mechanisms with respect to their ability to explain the observed data. This could be done for example using sufficiently detailed Dynamical Causal Models and Bayesian model comparison [35]. If neither intervention nor formal model comparison are feasible, the researcher would have to limit the interpretation of observed CFC patterns (see Figure 4) to that of a marker. This hierarchy of approaches is also reflected in the arrangement of methods in Figure 4.

To further exemplify the hierarchy of approaches illustrated in Figure 4, we turn to a different, more established measure — spectral power analysis. For example, measures of LFP power in the gamma band can simply be used as markers to classify different conditions (lower left corner in Figure 4b). However, we also have several reasonable biophysical models (conceptual and computational) about the generating mechanisms of hippocampal and cortical gamma oscillations. These mechanisms were ultimately identified by interventional approaches (pharmacological, genetic, lesion) in a variety of physiological systems, as well as in computational models [36]. This means the field of spectral power analysis can draw on interventional approaches as well as formal model comparisons (upper row/right column of Figure 4b). Nevertheless, we note that even for the mature field of spectral power analysis a change in LFP power in the gamma band can be due to several biophysical mechanisms (change in the number of neurons engaged in oscillations, their synchrony, etc.) and these are not mutually exclusive. However, we can still map changes in gamma activity to a limited number of mechanistic options, each of them relatively well understood. Furthermore, since we are aware of several alternative explanations such as eye-movement artifacts that might contribute to gamma-band power changes we can design experiments to control for them [37]. We believe that similar steps will be required before CFC patterns in a signal can be confidently linked to any concrete biophysical mechanism.

Practical recommendations

As previously discussed we will need progress in several directions to establish phase–amplitude or other types of CFC as a fundamental mechanism in coordinating neuronal activity. Together with experimental and modeling advances, stricter standards in the use of CFC metrics are also necessary. Below we list practical recommendations to avoid some of the mentioned confounds and increase the specificity of the most popular phase–amplitude CFC metric (see Figure 1). Rather than a comprehensive algorithm this list should be thought as a checklist that should help to minimize technical pitfalls and over-interpretation of phase–amplitude CFC measures in macroscopic signals.

1 *Presence of oscillations*. Signatures of oscillatory processes with clear peaks in a time-resolved power spectrum are indispensable prerequisites. The frequency component for defining the instantaneous phase should include one of the peaks.
2 *Selection of bandwidths*. The frequency band used to define the instantaneous phase should isolate energy associated with the oscillatory component of interest. If the center frequency is relatively stable a natural choice for the bandwidth can be directly obtained from the width of the corresponding peak in the power spectrum. The latter can be estimated by subtracting from the real power spectrum the power spectrum of baseline or a fit of the background power spectrum [38]. Note that the band defining the instantaneous amplitude at the higher frequency must be large enough to fit the sidebands caused by the assumed modulating lower frequency band (Figure 2a) and the lower frequency band should be narrow enough to define a meaningful phase. Therefore, adaptive rather than fixed bandwidths might be necessary when scanning the modulating frequency in explorative analyses.
3 *Interpretation of instantaneous phase*. A meaningful interpretation of instantaneous phase requires its monotonic growth in time. The presence of phase slips or reverses (also observed as negative instantaneous frequencies) must be checked and justified.
4 *Precision*. The precision of the method used to assign an instantaneous phase and amplitude to a signal should be determined for each analysis. The precision of the computation of the Hilbert transform of a signal $s(t)$ can be estimated from the variance of $s(t) + H^2(s(t))$, which analytically should be identical to zero. Given the non-locality of Hilbert (or wavelet) transforms, edge effects can be severe. It is recommended to discard at least a few characteristic periods of the signal at the beginning and end of each segment of interest.
5 *Testing for non-linearities*. Non-linear responses to input or nonlinearities during the signal transduction can contribute to phase–amplitude CFC. The presence of harmonics in the signal should be tested by a bicoherence analysis and its contribution to CFC should be discussed. Partialization of phase–phase and amplitude–amplitude coupling is necessary to assess the role of non-linearities in generating spectral correlations (see *Supplementary results: Atmospheric noise shows CFC after squaring the signal; Small static non-linearity in ECoG data generates CFC; Mathematical example of a non-linearity; Supplementary discussion: The transitivity of correlation between phase and amplitude*).
6 *Testing for input-related non-stationarities*. When the timing of neuronal input to the recorded area is available, an analysis of relative locking between phase, amplitude and input can inform about the origin of the correlations.
7 *Temporal structure*. Information about the temporal structure of the putative interaction (e.g. sustained during many cycles versus a transient coupling) can be helpful to better characterize a presumed CFC and to disambiguate its origins. The modulation index only offers an average measure of CFC by computing the distance from a phase–amplitude histogram to a uniform distribution. However, such histogram can be used to identify the phase at which the average amplitude of high-frequency activity is maximal. The time series obtained by sampling the amplitudes of

high-frequency activity at that particular phase can be used to provide some information about the temporal dynamics of the coupling.

8 *Surrogates*. Surrogate data should be created that minimally interfere with the phase and amplitude dynamics. For continuous recordings random point block-swapping is preferred over phase scrambling or cutting at several points.

9 *Specificity of effects*. Differences of CFC indices across conditions should be controlled for the differences in power at the presumed bands of interaction. The specific role of the coupling can be better assessed once the explanatory level of the power spectrum has been accounted for: When trial-based measures are available, stratification techniques should be used to compare subsets of trials for which the distributions of power at the bands of interest are identical across the conditions.

Conclusions

CFC might be a key mechanism for the coordination of neural dynamics. Several independent research groups have observed CFC and related it to information processing, most notably to learning and memory [4–7,8••]. Recently, CFC has also been used to investigate neurological and psychiatric disorders [9,10,11•,12–15]. Thus, CFC analysis is potentially a promising approach to unravel brain function and some of their pathologies.

In the present manuscript we have reviewed some confounds that hamper phase–amplitude CFC analysis. Importantly, these confounds have not been considered in a significant percentage of recent publications and may have contributed to over-interpretations. This is a serious issue that needs to be resolved because CFC analysis is potentially a powerful tool to reveal fundamental features of neural computations. An obvious first step is to adopt stricter standards and canonical procedures for CFC analysis. To this end we suggested a — probably incomplete — list of controls that should be routinely checked. We have also attempted to organize the current modeling/statistical approaches to CFC in order to better identify their respective advantages and pitfalls and to point out where further methodological advances are required. We close by suggesting to always use the term 'cross frequency correlation' instead of 'coupling', unless coupling is unequivocally demonstrated.

Conflict of interest statement

Nothing declared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.conb.2014.08.002.

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