Measuring phase-amplitude coupling between neuronal oscillations of different frequencies

Adriano B. L. Torta,*, Robert Komorowskib, Howard Eichenbaumb, Nancy Kopellc

aEdmond and Lily Safra International Institute of Neuroscience of Natal & Federal University of Rio Grande do Norte, Natal, RN 59066, Brazil; bCenter for Memory & Brain, Boston University, Boston, MA 02215, USA; cCenter for BioDynamics and Department of Mathematics and Statistics, Boston University, Boston, MA 02215, USA.

Abstract

Neuronal oscillations of different frequencies can interact in several ways. There has been particular interest in the modulation of the amplitude of high frequency oscillations by the phase of low frequency oscillations, since recent evidence suggests a functional role for this type of cross-frequency coupling (CFC). Phase-amplitude coupling has been reported in continuous electrophysiological signals obtained from the brain at both local and macroscopic levels. In the present work, we present a new measure for assessing phase-amplitude CFC. This measure is defined as an adaptation of the Kullback-Liebler distance - a function that is used to infer the distance between two distributions - and calculates how much an empirical amplitude distribution-like function over phase bins deviates from the uniform distribution. We show that a CFC measure defined this way is well suited for assessing the intensity of phase-amplitude coupling. We also review seven other CFC measures; we show that, by some performance benchmarks, our measure is especially attractive for this task. We also discuss some technical aspects related to the measure, such as the length of the epochs used for these analyses and the utility of surrogate control analyses. Lastly, we apply the measure and a related CFC tool to actual hippocampal recordings obtained from freely moving rats, and show for the first time that the CA3 and CA1

*Corresponding author
Email address: tort@natalneuro.org.br (Adriano B. L. Tort )
regions present different CFC characteristics.

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**Introduction**

Neuronal oscillations of different frequencies can interact with one other (Jensen and Colgin, 2007). The interaction of rhythms in different bands is commonly called “cross-frequency coupling” (CFC), and has been reported in continuous electrophysiological signals obtained at different levels, ranging from local, to more mesoscopic and macroscopic scales, as assessed by intracellular, local field potential (LFP), electrocorticogram and electroencephalogram recordings (Bragin et al., 1995; Lakatos et al., 2005; Canolty et al., 2006; Demiralp et al., 2007; Jensen and Colgin, 2007; Kramer et al., 2008; Cohen, 2008; Young and Eggermont, 2009). In one type of interaction, known as phase-amplitude coupling or nesting, the amplitude of high-frequency oscillations is modulated by the phase of low-frequency rhythms. Perhaps the best known example of this type of CFC occurs in the hippocampus, where theta (5 - 10 Hz) phase modulates gamma (30-100 Hz) amplitude (Bragin et al., 1995). Theoretical work suggests that such theta-gamma nesting plays a role in sequential memory organization and maintenance of working memory (Lisman and Idiart, 1995; Lisman, 2005).

Phase-amplitude coupling between neuronal oscillations has been receiving increasing interest, with a recent outpouring of papers in the last 5 years (Lakatos et al., 2005; Canolty et al., 2006; Demiralp et al., 2007; Hentschke et al., 2007; Jensen and Colgin, 2007; Kramer et al., 2008; Cohen, 2008; Lakatos et al., 2008; Tort et al., 2008, 2009; Cohen et al., 2009a,b; Schroeder and Lakatos, 2009; Young and Eggermont, 2009; Handel and Haarmeier, 2009; Wulff et al., 2009; Axmacher et al., 2010). Phase-amplitude CFC has been reported in species such as mice (Buzsaki et al., 2003; Hentschke et al., 2007; Wulff et al., 2009), rats (Bragin et al., 1995), sheep (Nicol et al., 2009), monkeys (Lakatos et al., 2005), and humans (Canolty et al., 2006; Cohen et al., 2009b,a; Axmacher et al., 2010), and in brain regions other than the hippocampus, such as the basal ganglia (Tort et al., 2008; Cohen et al., 2009a) and the neocortex (Lakatos et al., 2005; Canolty et al., 2006; Cohen et al., 2009b). New evidence supports the idea that this type of coupling presents a functional role in the execution of cognitive functions (Lakatos
et al., 2008; Tort et al., 2008, 2009; Cohen et al., 2009a,b; Axmacher et al., 2010), in accordance to theoretical models (Lisman, 2005). In particular, phase-amplitude coupling has been suggested to be involved in sensory signal detection (Handel and Haarmeier, 2009), attentional selection (Schroeder and Lakatos, 2009), and memory processes (Tort et al., 2009; Axmacher et al., 2010).

Several methods exist for assessing phase-amplitude coupling, and no single method has been chosen as the gold standard for detecting the phenomenon. The different measures possess different advantages and limitations and may be employed to serve different purposes (see Cohen 2008 and present results). Here, we describe in detail a measure that we have employed in recent publications for detecting phase-amplitude coupling (Tort et al., 2008, 2009). We also show how this measure compares with other measures, and, based on the results, we argue that the measure we propose has properties that make it attractive for quantifying the intensity of the nesting. Finally, we present a technique related to the measure - the phase-amplitude comodulogram - which scans multiple frequency pairs searching for CFC; as an example of its application, we apply the tool to analyzing in vivo hippocampal recordings in rats performing a cognitive task, and we demonstrate that the CA3 and CA1 regions may present different subbands of gamma oscillations modulated by theta phase.

Method Description

In this section we describe how our phase-amplitude CFC measure is computed, and the rationale for its definition. To be consistent with previous reports (Tort et al. 2008, 2009), we call the measure the modulation index (MI). The MI is able to detect phase-amplitude coupling between two frequency ranges of interest: the “phase-modulating” and “amplitude-modulated” frequency bands. We refer to these two frequency bands as the phase \((f_p)\) and amplitude \((f_A)\) frequencies, respectively. Below we describe the steps required for the computation of the MI.

Amplitude and phase time series extraction and the construction of the phase-amplitude plot

We denote by \(x_{raw}(t)\) the raw signal (e.g., the unfiltered LFP). The MI is calculated from a phase-amplitude distribution-like plot (Figure 1), which is obtained as follows:
1) First $x_{raw}(t)$ is filtered at the two frequency ranges under analysis ($f_p$ and $f_A$). We denote the filtered signals as $x_{f_p}(t)$ and $x_{f_A}(t)$.

2) The time series of the phases of $x_{f_p}(t)$ (denoted as $\phi_{f_p}(t)$) is obtained from the standard Hilbert transform of $x_{f_p}(t)$. The Hilbert transform is also applied to $x_{f_A}(t)$ to extract the time series of the amplitude envelope of $x_{f_A}(t)$ (denoted as $A_{f_A}(t)$). The composite time series $(\phi_{f_p}(t), A_{f_A}(t))$ is then constructed, which gives the amplitude of the $f_A$ oscillation at each phase of the $f_p$ rhythm.

3) Next, the phases $\phi_{f_p}(t)$ are binned, and the mean of $A_{f_A}$ over each phase bin is calculated. We denote by $<A_{f_A}>_{\phi_{f_p}}(j)$ the mean $A_{f_A}$ value at the phase bin $j$.

4) Lastly, we normalize the mean amplitude $<A_{f_A}>_{\phi_{f_p}}$ by dividing each bin value by the sum over the bins:

$$P(j) = \frac{<A_{f_A}>_{\phi_{f_p}}(j)}{\sum_{j=1}^{N} <A_{f_A}>_{\phi_{f_p}}(j)},$$

where $N$ is the number of phase bins\(^1\). Note then that the normalized amplitude $P$ has the same characteristics as a discrete probability density function (pdf); that is: $P(j) \geq 0 \ \forall \ j$ and $\sum_{j=1}^{N} P(j) = 1$. Although $P$ is not defined from a random variable - in contrast to the classical definition of a pdf -, we will refer to this distribution-like function as the “amplitude distribution”. The phase-amplitude plot is obtained by plotting $P$ as a function of the phase bin. In Figure 1 we show this procedure outlined above for a synthetic signal using theta as $f_p$ and low gamma (LG, 30-60 Hz) as $f_A$.

Note that the intensity of the phase-amplitude coupling can be already inferred by visual inspection of the phase-amplitude plot\(^2\); however, it is desirable to extract a number out of a plot like this that corresponds to the

\(^1\)We have been typically employing $N = 18$, i.e., we bin the 0° to 360° interval into eighteen 20° intervals. However, we note that in some circumstances a higher number of bins might be desirable (e.g., when working with multimodal distributions; see Figure 13).

\(^2\)In this work, we do not define a particular CFC measure (among the ones we review) as being the gold standard for assessing the level of phase-amplitude coupling. Therefore, we often ask the reader to intuitively infer the level of coupling by either the visual inspection of the amplitude envelope, or, equivalently, of the phase-amplitude plots. In particular, in the present work, example cases presenting identical phase-amplitude plots are said to possess the same levels of phase-amplitude coupling.
intensity of the coupling. This is what our MI measure does, as explained below.

**Rationale for the definition of the Modulation Index**

Note that if there is no phase-amplitude coupling between the pair of frequencies \((f_p, f_A)\) under study, the amplitude distribution \(P\) (defined above) over the phase bins is uniform, i.e., on average, the amplitude of \(f_A\) is the same for all phases of the \(f_p\) oscillation. The existence of phase-amplitude coupling is characterized by a deviation of the amplitude distribution \(P\) from the uniform distribution in a phase-amplitude plot. Following this reasoning, we defined a measure that quantifies the deviation of \(P\) from the uniform distribution. This was achieved by an adaptation of the Kullback-Leibler (KL) distance, a premetric that is widely used in statistics and in information theory to infer the amount of difference between two distributions (Kullback and Leibler, 1951). The adaptation we performed was simply to make the distribution distance measure assume values between 0 and 1. Our MI is therefore a constant times the KL distance of \(P\) from the uniform distribution.

In terms of equations, the KL distance of a discrete distribution \(P\) from a distribution \(Q\) is defined as:

\[
D_{KL}(P, Q) = \sum_{j=1}^{N} P(j) \log \left( \frac{P(j)}{Q(j)} \right)
\]

The KL distance has the property that \(D_{KL}(P, Q) \geq 0\) and \(D_{KL}(P, Q) = 0\) if and only if \(P = Q\), i.e., when the distributions are the same.

Notice that the KL distance formula resembles the definition of the Shannon entropy \((H)\) of a distribution \(P\), which is given by

\[
H(P) = -\sum_{j=1}^{N} P(j) \log(P(j))
\]

In fact, the KL distance is related to the Shannon entropy by the following formula:

\[
D_{KL}(P, U) = \log(N) - H(P)
\]

where \(U\) is the uniform distribution. Notice further that \(\log(N)\) is the maximal possible entropy value, which happens precisely for the uniform distribution (when we have \(P(j) = 1/N\) for all bin \(j\)). Therefore, as \(H(P) \leq \log(N)\),
we defined our MI by dividing the KL distance of the observed amplitude distribution ($P$) from the uniform distribution ($U$) by $\log(N)$:

$$MI = \frac{D_{KL}(P,U)}{\log(N)}$$

Therefore, if the mean amplitude is uniformly distributed over the phases (i.e. $P = U$, meaning lack of phase-amplitude coupling), we have $MI = 0$; $MI$ increases the further away $P$ gets from $U$ as inferred by the KL distance. A $MI$ value equals to 1 happens if $P$ is a Dirac-like distribution, that is $P(k) = 1$ for a given bin $k$ and $P(j) = 0$ for all bins $j$ different than $k$. This would denote an oscillation $f_A$ that just exists in a single phase bin of $f_p$ and vanishes at the other phase bins.

In Figure 2, we show the performance of the MI in assessing different cases of phase-amplitude coupling, using theta-nested gamma as example. Note that the MI tracks the intensity of the coupling, as seen more intuitively from the amplitude distributions.

**Dependence of the MI on the data length**

In this section we discuss the dependence of the MI on the length of the signal. If a given brain signal is a perfect periodic function possessing no noise component, the amount of phase-amplitude CFC can be inferred from very short epochs of the data, as long as the $\phi_{f_p}(t)$ and $A_{f_A}(t)$ time series are longer than a full cycle of the $f_p$ rhythm. However, given that virtually all continuous signals recorded from the brain include a significant amount of noise, a natural question that arises is how long the analyzed epoch should be in order to average out the noise component.

We have investigated this issue using synthetic LFPs in which we could control the length of the signal and the intensity of noise while preserving the amplitude envelope and all other parameters of the LFPs (see Appendix); this preserves what we intuitively think of as the parameters that produce the CFC. The results of this analysis are shown in Figure 3. We ran 100 trials for each set of parameters under study (epoch length and noise intensity); we found that longer epochs led to smaller MI variation among trials, as inferred from the coefficient of variation (CV) (Figure 3A). We also found that, in general, a higher intensity of coupling is associated with lower CV for the same epoch length (Figure 3B). This means that strong coupling can be more confidently inferred than weak coupling for short time epochs.
Note that even after defining an acceptable level for the variation in the measurements (e.g., assuming that a reliable measurement is associated with a CV of 10% or less), we are unable to recommend a universal minimal epoch length to be used in all experimental settings, as the measurement is also dependent on the amount of noise present in the signal (Figure 3A), which may vary among different data sets and laboratories. We also note that the minimal data length for a reliable measurement is dependent on the $f_p$ frequency, as slower oscillations will have less cycles sampled than faster oscillations. Using LFPs and studying theta as $f_p$, we have been typically computing the MI for epochs longer than 30 s (i.e, > 200 cycles analyzed), since we found this length long enough to provide us with a reliable measurement in our experimental setting. We recommend that each laboratory should perform its own control analysis to assess the minimal data length providing a reliable measurement (we note that this issue pertains to all CFC measures, not only the MI). In some cases, however, the nature of the paradigm employed does not allow long epochs to be analyzed; to circumvent this, one can make use of surrogate control analyses to evaluate whether the measured MI can be explained by random fluctuations in the signal or else if it denotes true coupling; this is the topic of the next section.

**Surrogate Control Analyses**

When analyzing experimental results, a statistical control analysis can be performed for a single MI value to infer whether the observed value actually differs from what would be expected from chance. Common in the analysis of neurophysiological data is the generation of a chance distribution derived from the analysis of surrogate time series that share statistical properties with the original data (Hurtado et al. 2004). To this end, the surrogate time series is usually obtained from a trial shuffling procedure, although alternative methods also exist.

In Figure 4, we show an example of such control analysis applied to an actual theta-LG phase-amplitude plot derived from *in vivo* LFP recordings; these were recorded from the CA3 region of the hippocampus while a rat was performing a task. For each trial of this task, the rat is allowed to explore its current spatial context (in order to perform a subsequent associative choice), and the resulting phase-amplitude plot was constructed using 20 trials in a session where the animal learned the task (see Tort et al. 2009 for details on data acquisition and task design). We created shuffled versions
of the \((\phi_{fp}(t), A_{fA}(t))\) time series by associating the phase series \((\phi_{fp}(t))\) of trial \(k\) with the amplitude series \((A_{fA}(t))\) of trial \(l\), with \(k\) and \(l\) randomly chosen among the trial numbers. We then generated 200 surrogate MI values, from which we could infer the MI chance distribution. As shown in Figure 4, the trial shuffling procedure breaks the appearance of phase-amplitude coupling, which is reflected by very low MI values (mean = \(1.43 \times 10^{-5}\), SD = \(1.12 \times 10^{-5}\)) compared to the measured MI value (\(88.29 \times 10^{-5}\)). Indeed, considering \(p < 0.01\) as significant, we find that the significance threshold lies on \(4.30 \times 10^{-5}\), much below the experimental result. We can therefore conclude that there was real (above chance) coupling between theta-phase and LG-amplitude in CA3 while the rat was exploring the arena in our paradigm.

As discussed above, the surrogate control analyses is particularly important when assessing the MI of short data epochs, as random fluctuations of the signal could give rise to artifactual coupling. With long data epochs, such as the one analyzed above, the noise influence is less likely to affect the MI, since the noise component gets averaged out from the phase-amplitude plot when a high number of cycles is analyzed.

**Comparison with other phase-amplitude coupling measures**

In this section, we quickly review other phase-amplitude coupling measures and compare their performances.

1) **The heights ratio**

A possible CFC measure is simply to compute the ratio:

\[
\frac{h_{\text{max}} - h_{\text{min}}}{h_{\text{max}}},
\]

where \(h_{\text{max}}\) and \(h_{\text{min}}\) are the maximal and minimal amplitude heights, respectively, inferred from the same phase-amplitude plot used to compute the MI (Figure 5A). We note that this measure has equivalent variations such as the “modulation ratio” employed by Lakatos et al. (2005) defined by \(h_{\text{max}}/h_{\text{min}}\), and also the ratio \((h_{\text{max}} - h_{\text{min}})/(h_{\text{max}} + h_{\text{min}})\) used in AM radio transmission, both of which provide qualitatively similar results (not shown). We opted to use the heights ratio as defined above because it is intuitive and bounded between 0 and 1.
2) *The power spectral density of the amplitude envelope*

As observed by Cohen (2008), the existence of phase-amplitude modulation can be assessed by analyzing the power spectral density (PSD) of the instantaneous amplitude time series $A_{f_A}(t)$ (Figure 5B). Notice that this measure has the advantage of fixing just the $f_A$ frequency, while multiple $f_p$ can be analyzed simultaneously. The occurrence of a peak in the PSD characterizes the existence of phase-amplitude coupling between $f_A$ and the frequency band(s) where the peak(s) occurred. The CFC intensity can be inferred by the integral (or mean) power over the phase-modulating band. In the examples depicted in Figures 1, 2 and 5, the theta-LG coupling strength is obtained by integrating the LG amplitude PSD over the theta band.

3) *The mean vector length*

Canolty et al. (2006) pointed out that a time series defined in the complex plane by $A_{f_A}e^{i\phi_{f_p}}$ could be used to extract a phase-amplitude coupling measure. In our example case of theta-LG coupling (Figures 1 and 2), each instantaneous LG amplitude point is represented by the length of the complex vector (i.e., the distance from the center (0,0)), whereas the theta phase of the time point is represented by the vector angle. In the case of an absence of phase-amplitude coupling, the plot of the $A_{f_A}e^{i\phi_{f_p}}$ time series in the complex plane is characterized by a roughly uniform circular density of vector points, symmetric around zero, as the $A_{f_A}$ values are on average the same for all phases $\phi_{f_p}$. If there is modulation of the $f_A$ amplitude by the $f_p$ phase, this means that $A_{f_A}$ is higher at certain phases than others. This higher amplitude for certain angles will lead to a “bump” in the complex plane plot of the $A_{f_A}e^{i\phi_{f_p}}$ time series, leading to loss of symmetry around zero. This loss of symmetry can be inferred by measuring the length of the vector obtained from the mean over all points in the complex plane. It is thus assumed that a symmetric distribution as it occurs during lack of coupling leads to a small mean vector length (as the points in the different phases would cancel each other), whereas the existence of coupling leads to a larger mean vector length (as the points in the “bump” would prevail over the others; see Figure 5C).

4) *The phase-locking value*

Penny et al. (2008) and Cohen (2008) have recently described new phase-amplitude CFC measures. One of these proposed measures has been named the *Phase-Locking Value (PLV)* and is defined by $PLV = ||<\exp(i(\phi_{f_p} - \phi_{A_{f_A}}))>||$, where $\phi_{A_{f_A}}$ is the phase time series of the amplitude envelope.
(see Figure 6), \(< >\) denotes the mean over all time points, and ||.|| calculates the length of the mean vector. By its definition, this measure provides the value of 1 whenever the phase series are locked and 0 if they are completely desynchronized (for a sufficiently large number of time points), and it constitutes a useful measure for detecting phase-amplitude coupling.

5) The correlation coefficient

Another measure reviewed by Penny et al. (2008) is based on the assessment of the correlation between \(A_{f_A}\) and \(f_p\) (or its normalization, defined by \(\cos(\phi_{f_p})\)):

\[
r_{ESC} = \text{Corr}(f_p, A_{f_A}),
\]

where \(ESC\) stands for envelope-to-signal correlation. In Figure 7 we show some examples of scatter plots between \(f_p\) and \(A_{f_A}\).

6) The general linear model

As observed by Penny et al. (2008), the correlation coefficient measure described above has the deficiency of being influenced by the phase of the coupling. In particular, as shown in Figure 7A, notice that this measure is not able to detect coupling at \(\pi/2\) phase-difference between the \(f_p\) and \(A_{f_A}\). In order to circumvent this, Penny et al. (2008) created a new measure based on a general linear model (GLM); this measure is basically a generalization of the correlation coefficient measure that is able to detect phase-amplitude coupling at all phase lags (see Penny et al. 2008 for details).

7) The coherence value

More recently, Colgin et al. (2009) have employed a phase-amplitude CFC tool based on the coherence spectrum between \(A_{f_A}\) and the original signal \(x_{raw}(t)\). This method is based on the same principle as the PLV measure, although it uses a different tool for assessing the level of phase-locking. As in the case of the amplitude PSD, a coupling measure can be defined by integrating the coherence levels over the phase-modulating band (Colgin et al., 2009).

**Performance Comparison**

Recent reports have suggested that the intensity of the phase-amplitude coupling may change depending on cognitive demands (Tort et al., 2008) and performance (Tort et al., 2009); it is therefore desirable to have a metric that is able to assess the magnitude of the coupling (c.f., Figure 2), in addition
to detecting its existence. As we will show below, the principles underlying four of the CFC measures reviewed above make them potentially not suitable for measuring CFC intensity, although these measures may present a positive correlation with coupling strength in a realistic scenario where noise is present in the system (see below).

Accordingly, the measures that are potentially not suitable to properly track the intensity of the coupling are the last four reviewed above: the two measures based on the levels of phase-locking between $A_{fa}$ and $f_p$ (the PLV and the coherence value) and the two measures based on the linear regression between $A_{fa}$ and $f_p$ (the correlation coefficient and the GLM). The explanation for the first case is the following: notice that different levels of phase-amplitude coupling can occur for the same level of phase-locking; the $\phi_{A_{fa}}$ time series will be exactly the same irrespective of the magnitude of the variation in the levels of the amplitude envelope $A_{fa}$ if the level of phase locking is the same. For instance, if $A_{fa}$ varies (phase-locked to $f_p$) between its maximal value and 90% of its maximal value or between the maximal value and 50% of its maximal value, the $\phi_{A_{fa}}$ series will be similar in both cases, varying from 0 to 360° (see Figure 6). Therefore, in principle, neither the PLV nor the coherence value - which depend essentially on phase-locking - can properly distinguish different levels of phase-amplitude coupling.

The two measures based on linear regressions are also potentially not suitable to assess different levels of coupling because the linear correlation between two variables $X$ and $Y$ is the same as between $X$ and $C \times Y + D$, where $C$ and $D$ are multiplicative and additive constants, respectively; notice that $D$ corresponds to a fixed fraction of the amplitude envelope that does not vary, and that different levels of coupling are reflected by different $D$ values and regression slopes ($C$), which do not change the correlation/regression coefficient (see Figure 7B).

In Figure 8 we show the results obtained for the eight measures during the analysis of similar phase-amplitude coupling cases as in Figure 2. For the same cases of coupling strength (as seen from the distributions shown in Figure 8A), we have analyzed the performance of the measures under two situations differing on the presence or not of noise in the synthetic LFP. In a situation without noise (Figure 8B), we confirmed the claims above that none of the measures based on the assessment of linear regression or coherence between $A_{fa}$ and $f_p$ correlates well with the CFC intensity (Figure 8B top row). On the other hand, all the other measures, namely the heights ratio, the mean vector length, and the amplitude PSD, were able to correlate with
the magnitude of the CFC, similarly to the MI (Figure 8B bottom row).

When analyzing synthetic LFPs with noise (the amplitude distributions of these cases are similar to Figure 8A, since the noise gets averaged out), we then obtained that all measures, including the regression- and coherence-based measures, were able to track the intensity of the coupling (Figure 8C). That is, although in principle four measures would not be able to correlate with CFC intensity, they end by doing so in a more noisy, realistic scenario. The reason for this is because higher levels of CFC intensity usually present a higher signal-to-noise ratio, being more easily identified than weaker cases of CFC. This is translated into a better linear regression/coherence between $A_{f_A}$ and $f_p$, explaining why the 4 measures discussed above are able to track coupling strength in the presence of noise despite the limitations intrinsic to their definition (Figures 6 and 7).

To explore this matter further, we have assessed the tolerance of the CFC measures to random fluctuations by increasing the white noise level of our synthetic signal. The results of this analysis are shown in Figure 9. As expected, we found that all regression- and coherence-based measures are very sensitive to noise, and the values they provide present a clear negative dependence on noise level (Figure 9 top row). We also found that all the other measures but the amplitude PSD present a good tolerance to noise (Figure 9 bottom row).

We next increased the amplitude of the LG oscillation in our synthetic LFP, while maintaining the level of coupling, and we verified that the mean vector length and the amplitude PSD measures are dependent on the absolute amplitude level of the amplitude-modulated band (Figure 10; see also Figure 11 for an intuitive explanation of this dependence). Although the regression- and coherence-based measures are insensitive to different levels of absolute amplitude in a situation without noise (Figure 10C), they do present a clear positive dependence on the absolute amplitude when noise is present in the LFP (Figure 10D); this result is readily explained by the fact that larger amplitudes are associated with a higher signal-to-noise ratio (c.f. discussion above). Only the MI and the heights ratio measure turned out to be completely independent on the absolute level of the amplitude-modulated band (Figure 10).

We also tested whether these measures would be able to detect the “width” of the modulation. As exemplified in Figure 12A, the amplitude peak as a function of the phase can have different widths, even for the same values of maximal and minimal amplitudes. We found that the MI and the amplitude
PSD seem more suitable for detecting these effects, followed by the mean vector length analysis (Figure 12B). Clearly, the heights ratio measure is unable to detect these effects by its very definition. We also found that the regression- and coherence-based measures do not perform well in detecting the modulation width (not shown).

Lastly, we tested the ability of these CFC measures in detecting multimodal amplitude distributions. We note that, by their definitions, the regression- and coherence-based measures do not perform well in cases of multimodality (as also observed by Penny et al. 2008), and we have therefore focused our analysis on the other measures. As shown in Figure 13, the heights ratio measure always assumes the same value irrespective of the existence of multimodality or not, as expected from its definition. The mean vector length and the amplitude PSD measures were unable to detect symmetric multimodal distributions (Figure 13A) (the PSD detects a coupling at twice the theta frequency in our example case). However, the latter two measures were able to detect phase-amplitude coupling in non-symmetric cases of multimodality (Figure 13B). On the other hand, the MI measure was able to detect both symmetric and asymmetric cases of multimodal distribution, and its values were sensitive to the different cases studied (Figure 13).

Based on the summary of these findings presented in Table 1, we conclude that the MI has some properties not shared by other measures that make it well suited for assessing the intensity of phase-amplitude coupling.

The Phase-Amplitude Comodulogram

Although the MI measure is able to examine only two frequency ranges at time [i.e., an \((f_p, f_A)\) pair], it can be used to construct a phase-amplitude comodulogram plot, a tool that reports the level of coupling among multiple bands simultaneously. The comodulogram is obtained by scanning frequency band pairs and applying the CFC measure to each one of them. Although computationally expensive, this analysis is ideal for searching for phase-amplitude couplings when no a priori assumptions are made about the phase-modulating \((f_p)\) and the amplitude-modulated \((f_A)\) frequency bands. The results are described using a pseudocolor plot that indicates the level of coupling between several narrowed-filtered frequency bands pairs. Typically, the abscissa represents the frequencies analyzed as \(f_p\), while \(f_A\) is represented in the ordinate axis; that is, hot colors in a given coordinate \((x, y)\) of
the bidimensional map indicate that the phase of the $x$ frequency modulates the amplitude of the $y$ frequency.

In Figure 14 we show an example of application of the comodulogram plot to actual in vivo hippocampal recordings. The comodulograms shown in this figure were constructed employing $f_p$ calculated in 2 Hz steps with 4 Hz bandwidths and $f_A$ in 5 Hz steps with 10 Hz bandwidths\(^3\). The data set analyzed and the task employed are the same as above (see Tort et al. 2009 for details). The comodulogram analysis was able to confirm the previously described (Bragin et al., 1995) theta-phase modulation of the amplitude of oscillations in the gamma range in the hippocampus during exploration (Figure 14). In addition, this analysis showed that theta-phase modulated more the low-gamma (LG, 30-60 Hz) sub-band in the CA3 region, while the high-gamma (HG, 60-100 Hz) sub-band was more modulated in CA1 (Figure 14).

Use of a common theta-phase signal (from a fissure electrode) indicated that the peak of CA1 HG amplitude precedes the peak of CA3 LG (Figure 14). This result therefore suggests for the first time different CFC characteristics between the CA3 and CA1 regions, while also questioning a monolithic definition for the gamma range as frequencies from 30 to 100 Hz. This example shows that new phase-amplitude CFC tools, such as the MI, and related techniques, such as the comodulogram, are able detect features about the brain rhythms that are blind to such standard tools as the Fourier analyses.

Discussion

We have presented a measure for assessing CFC of the phase-amplitude type. This measure (called MI) is defined as an adaptation of the KL distance, a function used to infer the distance between two distributions. Essentially, the MI is obtained by measuring the distance of an empirical amplitude distribution-like function over phase bins from the uniform distribution, which characterizes the absence of phase-amplitude coupling. We performed comparisons of the MI with other CFC measures, and we showed that, by some performance benchmarks, the MI is well suited for assessing the intensity of the coupling. Finally, we have applied the MI and related techniques to actual hippocampal recordings obtained from freely moving rats; these

\(^3\)That is, the $f_p$ ranges studied were $[0 \text{ Hz}, 4 \text{ Hz}]$, $[2 \text{ Hz}, 6 \text{ Hz}]$, $[4 \text{ Hz}, 8 \text{ Hz}]$, etc, while the $f_A$’s were $[10 \text{ Hz}, 20 \text{ Hz}]$, $[15 \text{ Hz}, 25 \text{ Hz}]$, $[20 \text{ Hz}, 30 \text{ Hz}]$, etc. The centers of these intervals correspond to coordinates in the comodulogram plot.
tools were able to detect previously described theta-gamma nesting in the hippocampus and also revealed characteristics about this coupling that were not known before.

Phase-amplitude coupling among brain rhythms has been receiving increasing interest, particularly because new findings are starting to link this phenomenon to the execution of cognitive functions (see Introduction). Along with the growth of interest in phase-amplitude CFC, there was a parallel development of new tools for studying these effects. In the present work, we have discussed 8 tools (including ours) that were recently developed. The different tools are based on different principles, and therefore each tool is suited to a specific purpose. For instance, the method devised by Cohen (2008) is strong at detecting transient coupling in short time epochs. Other measures, such as the heights ratio and the amplitude PSD, possess the advantage of being straightforward, which is likely to help convince readers of the findings reported.

A main limitation of the heights ratio measure however is that it takes into account only the amplitude information present in two phase bins (the ones with the maximal and minimal heights) while discarding the mean amplitude values in the other phase bins. This makes this measure unable to detect the width of the modulation; that is, for the same maximal and minimal heights, there can be a narrow or a wide modulation around the maximal height (Figure 12). This measure will also not distinguish unimodal from multimodal cases of phase-amplitude coupling, as, for instance, in a bi-phasic amplitude distribution (Figure 13).

The amplitude PSD, on the other hand, turned out to be very sensitive to noise (Figure 11). This sensitivity is readily explained if one considers that adding white noise to a signal is equivalent to adding a constant value to its PSD. In fact, the amplitude PSD has the important caveat of depending on the absolute amplitude of $f_A$: for the same level of phase-amplitude coupling (as inferred by the heights ratio, for instance), the integral of the PSD will be higher for higher $f_A$ amplitudes (Figure 10). This caveat also hinders the comparison of CFC intensities among different bands, as the different rhythms [e.g., low (30-60 Hz) and high (60-100 Hz) gamma] have different amplitudes, usually following a $1/f$ law. We found that normalizing and expressing the PSD as relative power (or “% Power”) makes it an inefficient phase-amplitude coupling measure for the purpose of quantifying the CFC intensity, although the normalized PSD is still able to detect the presence of coupling (not shown).
We also make two observations about the mean vector length measure: (1) a “small” or “large” mean vector length is a relative concept that depends on the $f_A$ amplitude. For instance, during the same presence (or absence) of coupling, the mean vector length will be higher for a higher $f_A$ amplitude (Figure 10). In another words, like the amplitude PSD measure, the mean vector length is also dependent on the absolute amplitude of the $f_A$ rhythm (Figures 9 and 10). This caveat however can be circumvented by working with normalized mean vector length measures, as performed by Canolty et al. (2006). (2) This measure provides low values when the $A_{f_A}e^{i\phi_{f_p}}$ time series is symmetric in the complex plane, as happens during the absence of coupling. However, a bimodal distribution of amplitude modulation can also produce cases that will not be detected by this measure (e.g., if the LG amplitude has two symmetric local maximums, one at the theta phase of $\phi$ and another at $\phi + \pi$, then the two amplitude “bumps” will mutually cancel each other in the mean vector analysis).

We have reviewed two measures based on linear regression and two measures based on the levels of phase-locking between $A_{f_A}$ and $f_p$ (namely the correlation coefficient, the GLM, the PLV, and the coherence value, respectively). We showed that something curious happens with these four measures: while in principle (see Figures 6 and 7) they should not be able to correlate with CFC intensity, they do present a positive correlation with CFC intensity in a realistic, noisy scenario. In fact, we have applied these measures to actual hippocampal LFP recordings, in which we have previously found increased theta-gamma coupling with learning (Tort et al., 2009), and we observed that these measures, similarly to the MI, were also able to track changes in CFC intensity (not shown). We concluded that differences in the signal-to-noise ratio associated to different coupling strengths likely underly these effects. Consistent with this, we found that these measures are very sensitive to the levels of noise present in the signal (Figure 9). Moreover, we found that these measures depend on the absolute amplitude of $f_A$ in the presence of noise (Figure 10); again, this dependence is likely explained by the fact that a higher $f_A$ amplitude is associated to a higher signal-to-noise ratio. In the light of these observations, we recommend care when analyzing results derived from the use of these techniques.

Recent reports have suggested that the strength of the phase-amplitude coupling may change depending on cognitive demands (Tort et al., 2008). It is therefore desirable to have a metric that correlates well with the intensity of the coupling. As we have stressed when reviewing the coupling measures,
there is a difference between being able to detect the phenomenon and to correlate with its intensity. We have devised our MI as a measure that is able to both detect and quantify the intensity of the coupling. We have relied on statistics and information theory to select a function that seemed suitable for this purpose: the KL distance. Though our measure is less intuitively understood, we believe this disadvantage is compensated by its performance (Table 1).

However, it should be remarked that we have here mainly studied the ability of these CFC tools in tracking the intensity of the coupling, as intuitively inferred by the visual inspection of phase-amplitude plots; though this parameter is of interest since it has been related to cognitive functioning (Tort et al., 2008, 2009), we note that this is a particular definition of “CFC intensity”, among others possible. Depending on the research protocol, one may be more interested in tracking the coherence between the amplitude envelope $A_{f_A}$ and the phase-modulating rhythm $f_p$, irrespective of the magnitude of the variations in $A_{f_A}$. In this case, both the coherence value and the PLV measures would be more appropriate measures than the MI. It should also be noted that the MI loses time information, and it cannot tell us whether the coupling occurred during the whole epoch being analyzed, or else if there occurred bursts of coupling inside the epoch. On the other hand, measures based on the coherence or the regression between $A_{f_A}$ and $f_p$ can give us a better idea of how consistent the coupling was in the whole epoch. Similarly, other CFC measures can be better suited than the MI for assessing other particular aspects of the data; assessing the CFC intensity - as defined in this work - is just one particular feature that can be studied in these signals.

We believe that the use of these new CFC tools should give rise to significant new findings in the coming years. In the present report we have provided an example of this, by showing that the CA3 and CA1 regions can have different sub-bands of gamma modulated by theta phase. Previously, we also reported novel high frequency oscillations (HFO) in the hippocampus that were detectable only by means of CFC analyses, and we showed that the amplitude of HFO and gamma oscillations can peak at different phases of theta (Tort et al., 2008). Such CFC tools give clues to the physiological underpinnings of the dynamics, and should facilitate the understanding of both the biophysical origins and the functions of brain dynamics.
Historical Notes, New and Classical References

References relevant to the MI measure include the classical paper by Shannon (1948) and the pioneer work by Kullback and Leibler (Kullback and Leibler 1951, see also Kullback 1959, 1987). More information on the Kullback-Leibler distance can be found in standard textbooks of Statistics and Information Theory, as well as in science encyclopedias online.

To the best of our knowledge, the MI equation employed by us was applied for the first time to Neuroscience research by Tass et al. (1998). In this work, the authors applied this index to measure the divergence of phase-difference distributions from the uniform distribution. Therefore, under their protocol, this measure was applied as a phase-phase coupling measure. The use of the MI as a phase-locking measure has been reviewed by Le Van Quyen et al. (2001), Hurtado et al. (2004), Young and Eggermont (2009), among others. The first application of the MI as a phase-amplitude coupling measure was done in Tort et al. (2008), where phase-amplitude distributions were analyzed. Notice that, as the MI is essentially a measure of distance from a uniform distribution, it has a wide applicability. What makes the MI specific to phase-phase coupling, or phase-amplitude coupling, or to any other effect under study, is the nature of the distribution being analyzed.

The analyses of multiple frequency pairs and expression of the results of a phase-amplitude CFC measure as a single bidimensional colormap (the “comodulogram”) was previously performed in Canolty et al. (2006) and Colgin et al. (2009) for their respective coupling measures, and in recent work of ours (Tort et al., 2008, 2009) for the modulation index described here.

Appendix

Our synthetic signal was modeled as:

\[
\begin{align*}
x_{\text{raw}}(t) &= A_{f_A}(t) \sin(2\pi f_A t) + \bar{A}_{f_p} \sin(2\pi f_p t) + W(t)
\end{align*}
\]

\footnote{The symbols \(f_p\) and \(f_A\) stand for frequency ranges throughout the main text; for parsimony of notation, however, in this Appendix section we employ these same symbols to denote fixed (single) numbers determining the theta and gamma frequencies in the synthetic LFP examples. Accordingly, we used \(f_p = 10\) Hz (theta) and \(f_A = 50\) Hz (LG) or 80 Hz (HG).}
where $W(t)$ is a Gaussian white noise process of variance $\sigma^2$, and $A_{f_p}$ is a constant determining the amplitude of $f_p$. In our simulations, the amplitude envelope $A_{fa}(t)$ of the “amplitude modulated” rhythm assumed different shapes, according to the different cases studied. In the cases of unimodal distribution, the amplitude envelope was defined as:

$$A_{fa}(t) = \bar{A}_{fa} \frac{(1 - \chi) \sin(2\pi f_p t) + 1 + \chi}{2}$$

where $\bar{A}_{fa}$ is a constant that determines the maximal amplitude of $f_A$, and $\chi \in [0, 1]$ is the fraction of the amplitude envelope that is not modulated by $f_p$; the parameter $\chi$ therefore controls the intensity of the coupling (notice that $\chi = h_{min}/h_{max}$ in the phase-amplitude plot; see Figure 5A).

For studying the width of the modulation, the amplitude envelope was modeled as follows:

$$A_{fa}(t) = \bar{A}_{fa} ((1 - \chi) g(f_p, t) + \chi)$$

where $g : \mathbb{R}^+ \times \mathbb{R}^+ \to [0, 1]$ is a normalized gaussian function defined by

$$g(f_p, t) = \frac{\Phi(s(f_p, t)) - \min(\Phi(s))}{\max(\Phi(s)) - \min(\Phi(s))}$$

with $\Phi$ denoting a normal distribution function with zero mean and variance $\hat{\sigma}^2$, and $s(f_p, t)$ is a sawtooth wave of frequency $f_p$. The width of the modulation can therefore be controlled by varying $\hat{\sigma}^2$.

The modeling of multimodal phase-amplitude distributions was done by employing a mixture of gaussian functions defined similarly as above, but presenting different phase lags in the sawtooth wave.

The MATLAB scripts used to compute the phase-amplitude plots and the MI can be obtained upon request to the authors.

Acknowledgments

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References


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Figure Legends

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