Neuroinformatics

ISSN 1539-2791/05/301–314/$30.00
DOI: 10.1385/NI:03:04:301

Original Article

Phase Synchronization Measurements Using Electroencephalographic Recordings

What Can We Really Say About Neuronal Synchrony?

Ramón Guevara,[1] *José Luis Pérez Velazquez,*[2] *Vera Nenadovic,*[3] *Richard Wennberg,*[4] *Goran Senjanović,*[1] *and Luís Garcia Dominguez*[*,2]

[1]Abdus Salam ICTP, Strada Costiera 11, 34100, Miaramare, Trieste, Italy; [2]Departments of Paediatrics and Institute of Medical Science, University of Toronto, Brain and Behaviour Programme and Division of Neurology, the Hospital for Sick Children, [3]Department of Critical Care, The Hospital for Sick Children, [4]Toronto Western Hospital, Krembil Neuroscience Centre, Division of Neurology, University of Toronto, Toronto, Canada

Abstract

Phase synchrony analysis is a relatively new concept that is being increasingly used on neurophysiological data obtained through different methodologies. It is currently believed that phase synchrony is an important signature of information binding between distant sites of the brain, especially during cognitive tasks. Electroencephalographic (EEG) recordings are the most widely used recording technique for recording brain signals and assessing phase synchrony patterns. In this study, we address the suitability of phase synchrony analysis in EEG recordings. Using geometrical arguments and numerical examples, employing EEG and magnetoencephalographic data, we show that the presence of a common reference signal in the case of EEG recordings results in a distortion of the synchrony values observed, in that the amplitudes of the signals influence the synchrony measured, and in general destroys the intended physical interpretation of phase synchrony.

DOI: 10.1385/NI:03:04:301

Index Entries: Electroencephalographic recordings; magnetoencephalographic recordings; phase synchronization; reference electrode; bipolar signal.

Introduction

The current interest in the determination of neuronal synchrony patterns in brain is derived from the modern conception of brain function, where synchronization of neuronal spike firing is felt to be a major determinant of the information processing capabilities of

*Author to whom all correspondence and reprint requests should be addressed.
E-mail: l.garcia.d@gmail.com

brain networks (Friston, 2001; Varela et al., 2001). Thus, the assessment of synchrony from electroencephalographic (EEG) recordings is being widely used in an attempt to unravel these basic mechanisms of brain function. However, a main concern when using EEG recordings is that the recording channels are bipolar: they record voltage differences at one electrode site relative to another electrode, normally also on the scalp. While this has been discussed by some (Shaw et al., 1979; Nunez, 1981), the situation is still unclear regarding what specifically can be misrepresented in phase synchrony analysis from these types of recordings. The limitations that a common reference electrode may impose when performing coherence analysis using EEG recordings have been addressed previously (Fein et al., 1988; Zaveri et al., 2000), indicating that any contamination in the reference channel will significantly affect the coherence measurements. While coherence and crosscorrelation methods have provided important insights in this regard (Nicolelis, 1999), the estimation of phase synchrony using the analytic signal concept (Gabor, 1946) has several advantages over the other more classical methods. Among these, and very crucial, is the benefit that the amplitude information can be separated from the phase, thus allowing strict determination of phase synchrony, without concerns about the instantaneous amplitudes of the signal.

In this work, we wish to submit the idea that this may not be the case when analyzing signals from voltage differences as in EEG, in which the amplitude information is still being considered. In addition, the time-varying signal recorded by the digital EEG common reference electrode during signal acquisition will significantly affect all phase synchrony measurements and therefore spurious synchrony may be detected. To illustrate the effect that the reference electrode has on the mean phase coherence (R), we have compared data from magnetoencephalographic (MEG) recordings (which do not use a reference electrode during acquisition) and EEG recordings obtained from epileptic patients. Using these comparative data as examples, we would like to stress how the results obtained by these methods of phase synchronization measurement, for EEG recordings in particular, must be interpreted very carefully.

Methods

Data Acquisition: EEG and MEG Recordings

In what follows, we will call "unipolar" a measurement or a signal that is reference free, like MEG, while bipolar will refer to those signals obtained by subtraction of two unipolar signals, a signal and its reference (i.e., EEG or the "simulated bipolar" MEG described below in this paragraph). MEG data were obtained from a whole-head 148 channel Canadian Thin Film (CTF) System (Port Coquitlam, Canada), from a patient with medically refractory frontal lobe epilepsy during the course of clinical investigations performed in the hope of identifying an epileptogenic focus amenable to surgical treatment. Data were acquired at a sampling rate of 625 Hz. For the purpose of this study, we examined a segment of 2 min with 20% overlapped windows of approx 3.5 s each. In each segment, the mean phase coherence (R) is calculated for both the raw MEG data and "simulated bipolar channels." Here a "simulated bipolar channel" refers to the signals obtained by subtracting two different unipolar (raw MEG) signals. As there are 148 channels for a given segment, we can obtain 10,878 R values corresponding to every possible pair. For the simulated bipolar channels, we have 10,878 × 146 R values, because for each pair we can form the corresponding bipolar channels after subtracting each one of the remaining channels (148 – 2 = 146).

EEG data were obtained from another patient with a history of temporal lobe epilepsy. Nineteen scalp EEG recording electrodes were applied according to the standard 10-20 International electrode placement system, plus a common reference acquisition electrode situated on the vertex between electrode positions F_z and C_z. The sampling rate in this case was 500 Hz. A 5-min segment was chosen for the analysis, with *R* evaluated for every 20% overlapped 4-s window. All signals were demeaned and bandpassed with an order 100 constrained least square finite impulse response filter (FIR-CLS) ($f \pm 2$ Hz) before the Hilbert Transform (HT) was applied. We explored frequency ranges from 3 to 50 Hz.

Data Analysis

Phase synchronization is defined as the degree of phase locking between two oscillators, phase locking being the condition where the phase difference

$$\Delta\alpha(t) = \alpha_n(t) - \alpha_m(t) \tag{1}$$

of the two oscillators *m*, *n* remains constant or nearly constant during a given period of time. $\alpha_n(t)$ denotes the instantaneous phase of signal *n*. The general condition should also include any multiples of the individual phases, but we will work here for simplicity with the relation 1:1 as stated in Eq. 1. Nevertheless our results are also valid for the general condition.

A common measure in the literature to determine the degree of phase locking is the mean phase coherence (*R*) (Mormann et al., 2000)

$$R = \left| \frac{1}{N} \sum_{j=1}^{N} e^{i\Delta\alpha(j\Delta t)} \right| \tag{2}$$

where $|\cdot|$ denotes absolute value and *N* is the number of data points that are being considered.

Before this measure can be applied we need to define the instantaneous phase (α) of a signal.

The instantaneous phase of a signal is defined as the instantaneous phase of its complex equivalent, the analytic signal. We obtain the analytic signal *w(t)* from a signal *s(t)* by

$$w(t) = s(t) + is_H(t) = A(t)e^{i\alpha(t)} \tag{3}$$

where $s_H(t)$ is the HT of $s(t)$ defined as

$$s_H(t) = \frac{1}{\pi} P \cdot V \int_{-\infty}^{+\infty} \frac{s(t)}{t-\tau} d\tau \tag{4}$$

Here $P \cdot V$ is the Cauchy principal value. The instantaneous phase of the analytic signal is then given by

$$\alpha(t) = \tan^{-1}\left(\frac{s_H(t)}{s(t)}\right) + k\pi \tag{5}$$

with $k = 0, \pm 1, \pm 2, \ldots$ If we consider two different real signals, s_n and s_m, we can find the phase difference between them as

$$\Delta\alpha(t) = \alpha_n(t) - \alpha_m(t) = \tan^{-1}\frac{s_{nH}(t)}{s_n(t)} - \tan^{-1}\frac{s_{mH}(t)}{s_m(t)} \tag{6}$$

An equivalent formulation is given in Eq. 9.

Two signals are synchronized if the temporal derivative of this phase difference is zero, that is, if the instantaneous frequencies coincide. Thus, taking the temporal derivative of the previous formula, the following condition must be satisfied

$$\frac{d}{dt}(\Delta\alpha(t)) = 0 \Rightarrow \frac{1}{1+\left(\frac{s_{nH}(t)}{s_n(t)}\right)^2} \frac{d}{dt}\left(\frac{s_{nH}(t)}{s_n(t)}\right)$$

$$= \frac{1}{1+\left(\frac{s_{mH}}{s_m}\right)^2} \frac{d}{dt}\left(\frac{s_{mH}(t)}{s_m(t)}\right) \tag{7}$$

In what follows, $s_{nx} = s_n - s_x$, will denote a bipolar real signal, the voltage difference between electrode n and x, where the (x) is the reference electrode; s_n denotes a unipolar real signal, while w_n stands for its analytic complex signal. Similar notation is used for analytic bipolar (e.g., $w_{nx} = w_n - w_x$). Note that, in EEG recordings, where one electrode always serves as a reference, we do not measure s_n but we infer it from s_{nx}.

Results

Analytic and Geometric Analysis

To understand how the application of phase synchronization methods via the HT may result in spurious synchrony findings due to the use of the reference electrode, we start by a simple analytical consideration of the problem. Let us assume that s_n and s_m are two synchronized signals such that equality (7) is valid. Take a third signal, say s_x, and call it the reference signal, as in the case of bipolar signal measurements. The question is: are the analytic signals w_{nx} and w_{mx} synchronized if w_n and w_m are synchronized between each other? The answer is, not necessarily. This can be seen by considering the phase differences between s_{nx} and s_{mx}. If those signals are synchronized, by using the linearity of the HT (e.g., $s_{nxH} = (s_n - s_x)_H = s_{nH} - s_{xH}$), and then substituting this new variable in Eq. 7 we obtain that the following relation should be satisfied:

$$\frac{1}{1+\left(\frac{s_{nH}-s_{xH}}{s_n-s_x}\right)^2}\frac{d}{dt}\left(\frac{s_{nH}-s_{xH}}{s_n-s_x}\right)$$

$$=\frac{1}{1+\left(\frac{s_{mH}-s_{xH}}{s_m-s_x}\right)^2}\frac{d}{dt}\left(\frac{s_{mH}-s_{xH}}{s_m-s_x}\right) \quad (8)$$

The point here is that the validity of Eq. 7 does not generally imply Eq. 8. In other words, if two signals are synchronized, their difference with a third signal are not in general.

We can show this graphically. In Fig. 1A we see two complex signals $w_n = s_n + is_{nH}$ and $w_m = s_m + is_{mH}$—in vector notation (s_n, s_{nH}) and (s_m, s_{mH})—and their respective phases (α_n, α_m) at a given moment t as well as their phase difference $\Delta\alpha$, at that moment. The complex signals w_n, w_m, and w_x are time-dependent vectors in the complex plane. The validity of the condition expressed in Eq. 7 implies that in order to maintain a constant phase difference (phase locking) these two vectors have to rotate at the same speed, or, equivalently, they need to have the same frequency. *Notice that there is no restriction to the amplitude variations of the vectors, all that matters is the relative rotation speed*. For "real life," noisy signals, this phase locking condition is evaluated statistically for a given time window, using R (Eq. 2) or alternative descriptions.

Let us now see how the bipolar signals and phases can be represented in this scenario. As the bipolar variables are the result of a signal subtraction with a reference signal and observing that the HT is linear, we can represent the bipolar vector w_{nx}—(s_{nx}, s_{nxH})—as the vector difference between w_n and w_x (the same for signal m). In Fig. 1B we depict the system at three different times, $t_0 < t_1 < t_2$. The bipolar vectors w_{nx} and w_{mx} are shown as thick lines, the unipolar vectors are shown as light lines, while dashed lines represent the unipolar reference vector w_x. *Note that the new phase differences between the two bipolar vectors will now be the βs and not the αs, this is in fact the key observation in this study*. We keep the symbol "α" for the unipolar phase difference. If w_n and w_m are synchronized, according to the definition, the angle α (previously called $\Delta\alpha$ in Fig. 1A) must be constant or nearly constant, while there is no restriction to the amplitudes. In Fig. 1, α will be kept constant from t_0 to t_2, ensuring phase synchronization between the unipolar vectors n and m. From t_0 to t_1 we rotate the unipolar vectors keeping the same angles but changing the norm of w_n,

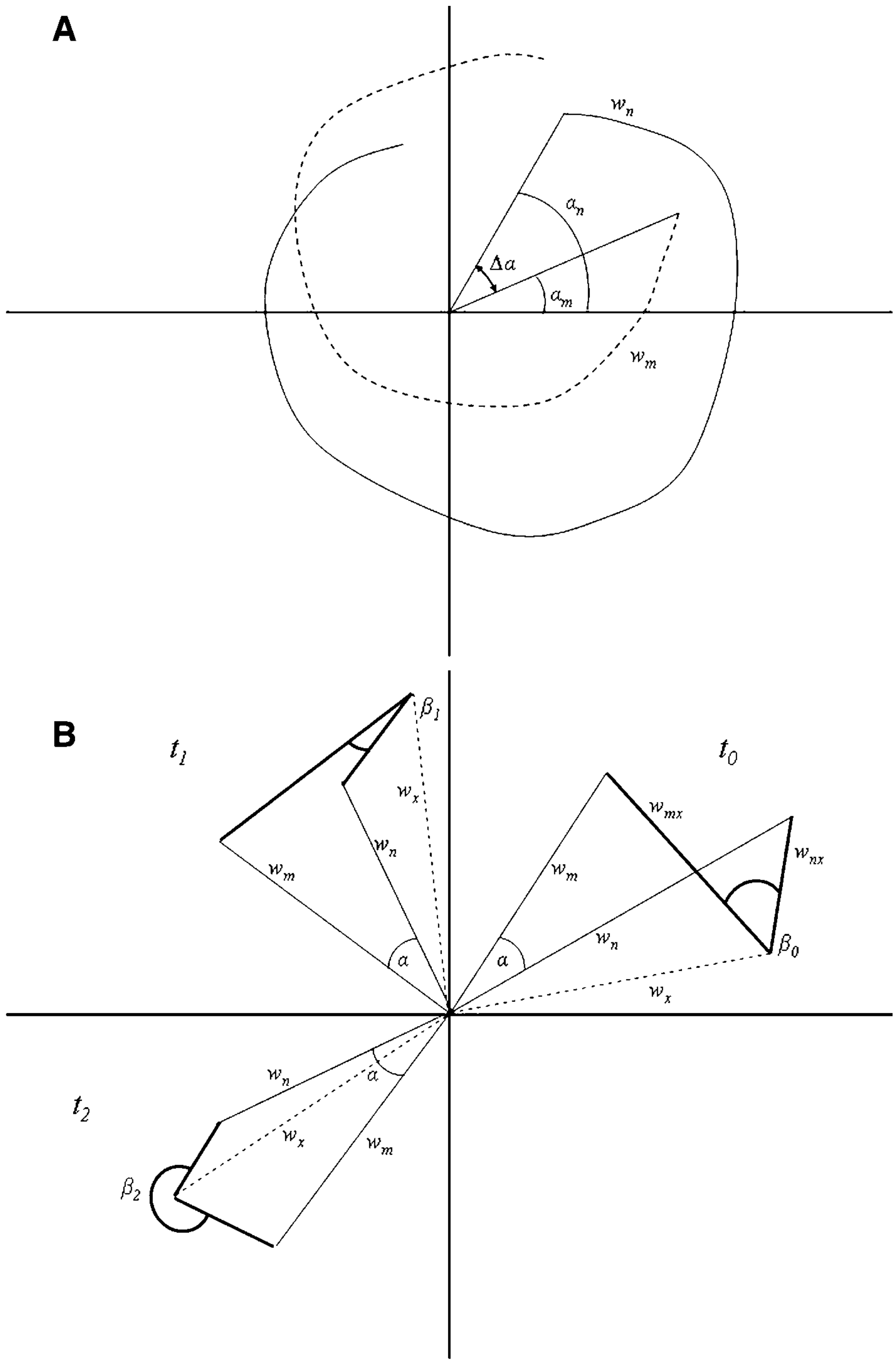

Fig. 1. Graphical representation of the analytic vectors on the complex plane. **(A)** Signals w_n and w_m (shown as two arbitrary traces rotating counterclockwise), their absolute phases (α_n and α_m) and their phase difference ($\Delta\alpha$). **(B)** Unipolar—w_m, w_n, and w_x—and bipolar—w_{mx} and w_{nx}—vectors, where w_x is the reference vector. The graph shows three different times—t_0, t_1, and t_2—during the rotation of the unipolar vectors. The angle α is the phase difference between the unipolar vectors (same as $\Delta\alpha$ in A) and β the corresponding for the bipolar signals.

making it smaller. This represents a change in the amplitude of the signal s_n. It can be seen that the angle between the bipolar vectors w_{nx} and w_{mx} will not remain constant ($\beta_0 \neq \beta_1$) indicating that those signals are not phase locked while, by construction, we know that w_n and w_m are in fact synchronized (we have not changed α). In general, we observe that the angle β, the phase we measure through bipolar vectors, can be greatly influenced by variations in amplitudes of the unipolar w_n, w_m, and w_x, thus invalidating the main supposed advantage of phase synchrony measures regarding the independence from amplitude variations.

From t_1 to t_2 we keep the same norm for the three vectors but we change the angle between w_x and w_n, w_m. While this does not influence the synchronization between w_n and w_m, it influences notably the synchronization we measure through w_{nx} and w_{mx}, again because of the corresponding change in the angle β.

From Eq. 6, the angle α can also be written as

$$\Delta\alpha = \tan^{-1}\frac{s_{nH}s_m - s_n s_{mH}}{s_n s_m + s_{nH}s_{mH}} \tag{9}$$

(*see* Appendix for details) while the corresponding expression for the bipolar angle β is

$$\Delta\beta = \tan^{-1}\frac{s_{nH}s_m - s_n s_{mH} - \left[s_{nH}s_x + s_{xH}s_m - s_{xH}s_n - s_x s_{mH}\right]}{s_n s_m + s_{nH}s_{mH} - \left[s_x s_m + s_n s_x - s_x^2 + s_{xH}s_{mH} + s_{nH}s_{xH} - s_{xH}^2\right]} \tag{10}$$

after the substitution and reordering of the terms. In Eq. 10 we note that up to the terms in brackets both equations are the same, and every new term added includes a contribution from w_x modulated or not by w_n and w_m. Thus, there is a complex interaction between all the three vectors and therefore all of these participate in the final estimated R. This analysis can also be extended to show the converse situation, that is, if two bipolar signals are phase locked their corresponding unipolars may not necessarily be phase locked.

Numerical Analysis Derived From EEG/MEG Recordings

To illustrate the effect of the aforementioned incongruence in real data, we now take recordings from human scalp EEG as well as reference-free MEG recordings. As the EEG is bipolar in nature and as the time-course of the voltage under the acquisition reference electrode is not known, we cannot actually identify the two component unipolar signals, but we can replace any component signal by another as we wish. From a given reference montage we can change the reference by simply subtracting the appropriate channels:

$$(S_n - S_x) - (S_m - S_x) = (S_n - S_m) \Rightarrow S_{nx} - S_{mx} = S_{nm}$$

where s_x has been substituted by s_m as a reference for s_n. In this way, we can change the reference electrode or transform (or "reformat") a reference montage to a bipolar† one. Here we will use this transformation to illustrate the effect that different references have on R. Figure 2 shows that the estimated R depends on the choice of the reference electrode.

Hence, the choice of the reference can considerably affect the synchrony values obtained, up to the point that they almost span the entire interval [0 1]. Note that, sometimes, a given reference tends to produce high synchrony values. That is the case in Fig. 2 with the reference x = 11 (symbol +, with most R values greater than 0.7). A close inspection of the raw data shows that this

†The word bipolar here refers to the so-called bipolar montage, where the word is used in a different sense from how we have used it so far. *See* Discussion section for details.

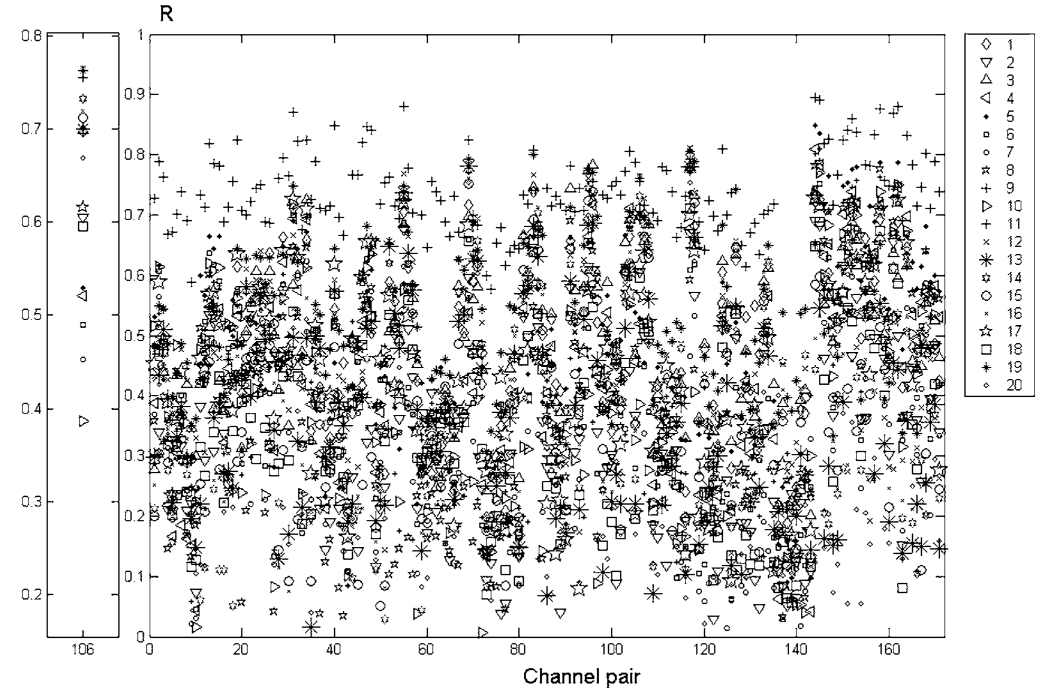

Fig. 2. *R* values using different reference electrodes *x*. Each symbol corresponds to a different reference being used, the *X* values stand for an indexing over the possible pairs of signals with the following order $\{s_{1x} - s_{2x}; s_{1x} - s_{3x}; s_{1x} - s_{4x}; \ldots s_{1x} - s_{19x}; s_{2x} - s_{3x}; s_{2x} - s_{4x}; \ldots s_{18x} - s_{19x}\}$. There are 171 different pairs in that list corresponding to 19 channels. For an any given pair, e.g., $X = 3$, we have 18 *R* values, corresponding to the synchrony between s_{1x} and s_{4x}, for $x = (2; 3; 5; 6; 7; \ldots; 20)$. $X = 20$ is reserved here for the original reference electrode. Note that lower case "*x*" refers to reference electrode while capital "*x*" refers to the values in the *X*-axis. The inset at the left shows pair 106.

channel has relatively high amplitude. It can be shown using the same geometrical argument for the case $\|w_x\| \gg \max\left(\|w_n\|, \|w_m\|\right)$ (where $\|w_n\| = \sqrt{s_n^2 + s_{nH}^2}$ is the norm or amplitude) that a high-amplitude reference will always produce high synchrony regardless of the time-course of w_n and w_m as long as these stay relatively small in amplitude. This can also be seen by substituting high values of s_{xH} and s_x in Eq. 8. On the other hand, if the norm of the reference tends to zero, then the results become closer to the real synchrony between w_n and w_m (check Eq. 8 or Fig. 1).

In order to show the influence of the w_x amplitude as well as the discrepancy between unipolar and bipolar *R*, we selected a segment of an MEG (148 channels) recording and applied the *R* measure to the raw data (unipolar in this case) and to every possible simulated bipolar channel. Figure 3 shows the *R* values obtained for simulated bipolar channels vs the real synchrony of those two channels as unipolar signals. We use every possible reference on a set of 10,878 pairs. The reference amplitude $\|w_x\|$ was changed six times by multiplying it by the numbers shown on the top of each graph. Note that high-amplitude real signals will produce high-amplitude analytic signals as the HT preserves the power spectra of the original signal.

As can be seen, there is a statistical dependence of the *R* values on the norm of the reference.

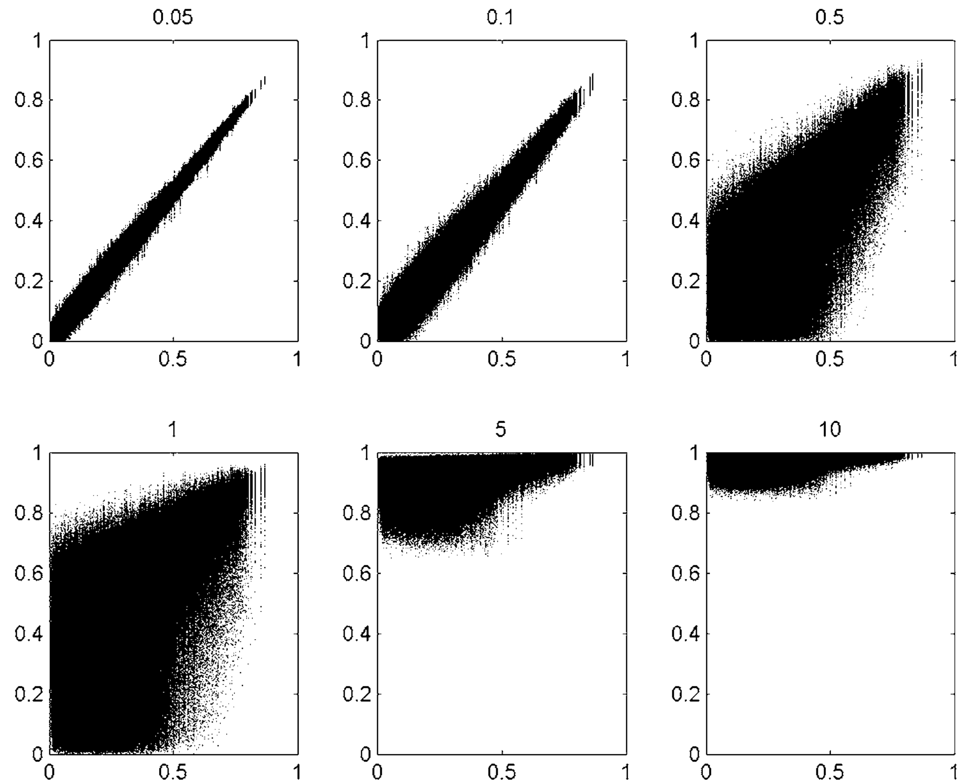

Fig. 3. Scatter plot of unipolar vs bipolar synchrony and the effect of the reference norm. *X*-axis shows *R* values of each pair in the raw magnetoencephalographic (MEG) data (unipolar), *Y*-axis shows the simulated bipolar synchrony for that pair after subtracting each one of the other 146 channels. As there are 148 MEG channels, there are 10,878 possible pairs and for each pair analyzed we use every one of the other 146 channels as reference. The number on the top of each graph is the relative amplitude of the reference channel. It can be noticed that the smaller the reference amplitude, the closer to the original (i.e., $x \approx y$) the *R* value estimated is.

As the reference tends to zero, its influence on the results also decays so that the calculated *R* calculated becomes closer to the real one: the points tend to stay closer to the line $y = x$. On the other hand, when the reference increases, the *R* values become higher and insensitive to the actual synchrony. In Fig. 3, the results were obtained from series not only demeaned but also normalized by the standard deviation (STD). This was done in order to construct the series with similar amplitude to show the effect in a clear way that the relative norm of the reference has on the *R* values; this transformation has no impact on the reference-free synchrony.

Averages have, in general, lower amplitude than single signals (if they all have the same mean value), and references can also be averages of two or more channels, as in the case of EEG recordings using, for example, linked ear lobes as the reference (probably the most common reference used in the literature). Therefore, we also tested to what degree the *R* values (obtained after an average reference is subtracted) approximate to the reference-free *R* values as the number of averaged channels increases. If the signals picked up by the electrodes are sufficiently independent, we can expect that the STD of these average signals will approximate to zero as

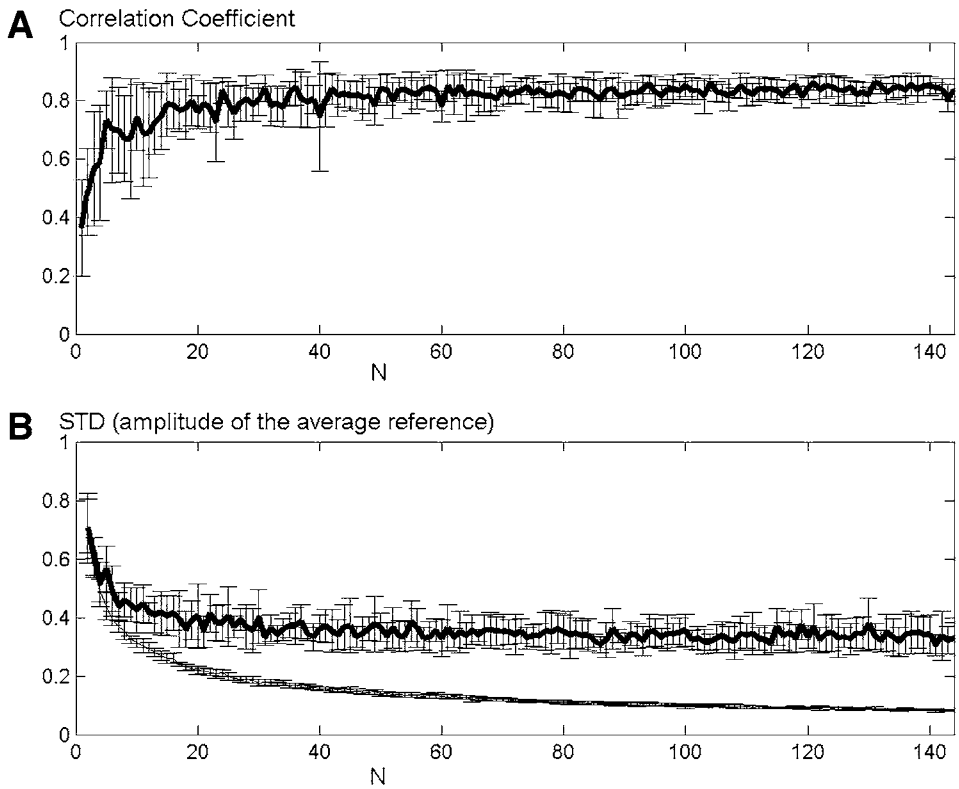

Fig. 4. (**A**) Correlation coefficient (for 60 randomly selected pairs of channels) between the *R* calculated with average references and the unipolar *R*. *N* is the number of averaged signals. The process was repeated 10 times and the vertical lines correspond to the standard deviation (STD) of the 10 samples calculated for each *N*. **(B)** Thick line, STD of averages; again vertical bars representing STD for a sample of 10. Light line, same procedure used for the previous line but applying a random shift to each signal considered in the average. This results in averages of time independent signals.

the number *N* of averaged channels increases. If this occurs, then the *R* values obtained with that nearly flat reference will be very close to the real, reference-free, *R*. We thus tested this on MEG signals and the results are presented in Fig. 4 Let us consider the references made of *N* channels from $N = 2$ to 144. For each *N*, we repeated the procedure 10 times, selecting each time *N* different channels at random. The reference *R* is then compared with the reference-free *R* using the correlation coefficient. Zero will indicate no correlation, while 1 will indicate that both *R* values are identical. In Fig. 4A, it can be seen that after averaging approx 20 signals, the correlation coefficient nearly stabilizes or increases very slowly in a value less than 1, i.e., between 0.80 and 0.85. At the same time, if we assess fluctuations in these averages, as measured by their STD, we see that these averages also decay very slowly (Fig. 4B, thick line). These averages stay "oscillating" to almost constant average amplitude indicating that there are intrinsic correlations in brain signals that cannot be avoided by considering more and more channels. This result can be compared with the one obtained from the same signals under the same procedure except for the application of random time shifting to avoid the presence of correlations (Fig. 4B, thin line). In this case, there is an evident negative slope whose

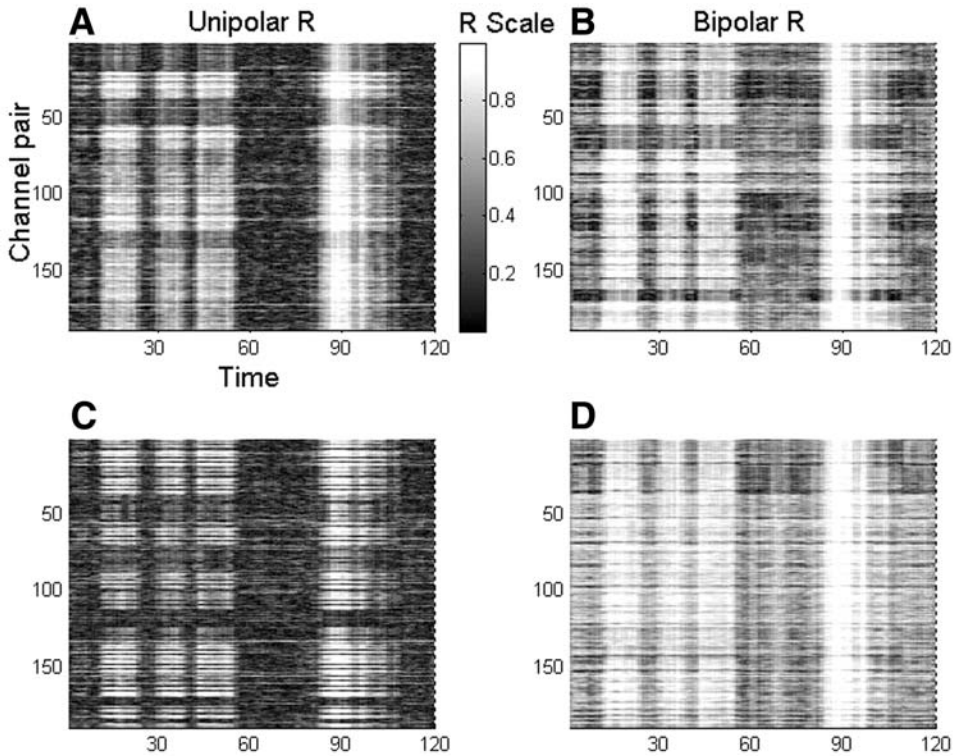

Fig. 5. *R* values for a 120-s segment of magnetoencephalographic (MEG) data in unipolar and corresponding simulated bipolar channels. Graphs corresponding to the same row (**A** and **B**, **C** and **D**) use the same channels.

extrapolation will go to a zero value of STD. High values in the STD ($N < 15$) in both the curves, at the beginning, are the result of random fluctuations because of the small sample size, while for $N > 15$ the constant STD (thick line) is reflecting intrinsic correlation in brain signals that do not strongly depend on N.

Figure 5 illustrates the time-course of R values for some sets of MEG channels in a continuous 120-s segment and its difference with some cases of simulated bipolar samples. Plots (A) and (C) are the R index in the raw MEG, (B) and (D) are the corresponding R values for the same channels after doing the subtraction with a given channel chosen at random, to simulate a bipolar recording. For each row, 20 different channels were chosen corresponding to 190 pairs.

The results presented in Figs. 2–5 were obtained for a central bandpass frequency of 35 Hz, but they do not depend on the central frequency used, as we checked this for frequencies ranging from 3 to 50 Hz, and obtained similar results.

Discussion

We have shown, using geometrical arguments and examples from real recordings, that the "choice" of a reference channel crucially determines the estimated synchronization patterns.

In EEG signals, we deal with voltage differences, but there are a number of different ways to choose the reference signal. In typical EEG reference montages, all voltage differences refer to one specific channel. In a so-called bipolar montage, the reference for every electrode is always different and normally spatially close to it. In reality, all EEG signals are referential: digitally "reformatted" bipolar EEG channels differ from the so- called reference montage channels only by virtue of the second electrode in each channel being situated much more closely to the first. Two other forms of EEG reference alternatives are the average and the Laplacian references. The last is the mathematical approximation to a reference-free signal (Hagemann et al., 2001). The results we obtained apply basically to the presence of a common reference: the reference montage and the average reference.

Moreover, it can be noted by using similar geometrical arguments that bipolar montages are subject to similar problems. Note that some particular channels in a bipolar montage are cases of common reference channels, for example F7-T3 and T3-T5 share the common reference T3.[§]

The results presented have been obtained for the case of the analytic signal approach via HT, but these limitations are also valid for the cases of Fourier and Wavelet descriptions, as they are all mathematically equivalent (under a suitable selection of the parameters) and produce equivalent results in practical applications (Le Van Quyen et al., 2001; Burns, 2004). The main recognized advantage of the phase synchrony analysis is that it captures the phase locking condition with total independence from the amplitude variations of the two oscillators. However, as we show here, the presence of a common reference destroys this property. Figure 1B shows, among other things, that when we apply a phase analysis to bipolar measures we are actually measuring phase locking in the not intended angle (β instead of α). It also shows that the synchrony calculation as applied to the instantaneous phase (angle β in Fig. 1B) is highly sensitive to amplitude variations for the three signals involved in the calculation as well as the relative phase movement of the reference.

It is important to note that the application of the habitual surrogate test for significance of synchrony analysis—randomly shifting the phases of the original signals—will not help us here in determining "significant" synchrony. In Fig. 2, we noted that high synchrony is detected when the reference electrode amplitude is relatively high. If a set of points in this graph shows a "significant synchrony," it should be those points with the highest values on the graph, which we already know are due to the reference amplitude. Thus, while possibly significant, the synchronization is artifactual and consequentially the surrogate analysis will mislead us in this case.

Also, the results shown here apply to any *n*:*m* frequency relation as well as lagged synchronization analysis (synchrony between series shifted in time). In the last case, the analysis is further distorted by the fact that the fixed reference will behave as a different reference for each signal as it is actually a shifted version of itself.

As the issue of synchrony is gaining importance in brain studies, there is an increasing number of reports dealing with applications of synchrony-based methods in EEG recordings (Chavez et al., 2003; Spencer et al., 2003). Most of these studies are based on EEG recordings with a common average reference. The common reference distorting the coherence measurements was directly addressed by Fein

[§]To avoid the shared reference problem, comparisons of bipolar channels without an electrode in common could be carried out, for example F7-T3 and T5-O1, but then one has no way to know which of the recording electrodes is most significantly active and the spatial meaning associated with any synchronization values is unclear (e.g., is it that the frontal [F7] region is synchronized with the occipital [O1], or is it that the mid-temporal [T3] region is synchronized with the posterior temporal [T5] region, and so on).

et al. (1988) and Zaveri et al. (2000), and has been commented upon by others (Nunez, 1981).

It is therefore conceivable that the variability reported in some studies may be largely related to the recording methods, in addition to the subject's cognitive state (Lutz et al., 2002). With these limitations in mind, one may be tempted to think that it could still be possible to use a common-referenced EEG for *relative comparisons under the same conditions*, for example, among subjects (provided the reference is maintained in the same place for all EEG recordings) experiencing different cognitive tasks (Rodriguez et al., 1999), or among patients, such as in studies addressing the synchrony patterns in epileptiform activity (Chavez et al., 2003). However, this is not the case, because, as we noted in Eq. 9 and 10, the R calculated from every pair in a referenced EEG is not just a functional distortion of real subjacent synchrony, but is also a function of a number of factors that have nothing to do with what R is supposed to measure. We consider that these kinds of studies should preferably be done using MEG or perhaps Laplacian EEG (provided a previous study is first done to confirm the theoretical suitability of the Laplacian technique for this type of analysis).

We also note that the choice of averaged references will not result in a significantly better approximation to the reference-free measure as the presence of intrinsic correlations, or common noise, will prevent the average signals from ever becoming flat. High values in the correlation coefficient in Fig. 4, for $N > 15$, might lead one to consider that using as reference the average of all channels in an EEG could result in a fairly good approximation. But this is not quite the case as the degree of long-range correlation in the brain can drastically change in different brain states, as in the case of seizures or even cognitive tasks, situations that are routinely addressed with this kind of analysis. These long-range correlations can produce averages that oscillate at high amplitude and as a consequence generate devastating effect in the calculated R.

In conclusion, a synchrony value obtained between two different sites in the brain from a common-referenced EEG recording is greatly affected by a number of factors that are currently considered not to play a role in this kind of analysis. As long as the reference signal is not constant, the calculated synchrony from these kind of recordings does not have a meaningful interpretation.

Acknowledgment

Our research is supported by a Discovery grant from the National Science and Engineering Research Council of Canada (NSERC).

References

Burns, A. (2004) Fourier-, Hilbert- and wavelet-based signal analysis: are they really different approaches? J. Neurosci. Methods, 137, 321–332.

Chavez, M., Le Van Quyen, M., Navarro, V., Baulac, M., and Martinerie, J. (2003) Spatio-temporal dynamics prior to neocortical seizures: amplitude versus phase couplings. IEEE Trans. Biomed. Eng. 50, 571–83.

Fein, G., Raz, J., Brown, F. F., and Merrin, E. L. (1988) Common reference coherence data are confounded by power and phase effects. Electroencephalogr. Clin. Neurophysiol. 69, 581–584.

Friston, K. J. (2001) Brain Function, Nonlinear Coupling, and Neuronal Transients Neuroscientist, 7, 406–418.

Gabor, D. (1946) Theory of Communications. J. IEE London 93, 429.

Hagemann, D., Naumann, E., and Thayer, J. F. (2001) The quest for the EEG reference revisited: a glance from brain asymmetry research. Psychophysiology 38, 847–857.

Le Van Quyen, M., Foucher, J., Lachaux, J., Rodríguez, E., Lutz, A., Martinerie, J., and Varela, F. J. (2001) Comparison of Hilbert transform and wavelet methods for the analysis of neuronal synchrony. J. Neurosci Methods 111, 83–98.

Lutz, A., Lachaux, J. P., Martinerie, J., and Varela, F. J. (2002). Guiding the study of brain dynamics by using first-person data: synchrony patterns correlate with ongoing conscious states during

a simple visual task Proc. Natl. Acad. Sci. USA 99, 1586–1591.
Mormann, F., Lehnertz, K., David, P., and Elger, C. E. (2000) Mean phase coherence as a measure for phase synchronization and its application to the EEG of epilepsy patients. Physica D 144, 358–369.
Nicolelis, M. A. L. (1999) Methods for Neural Ensemble Recordings, CRC Press, Boca Raton, FL.
Nunez, P. L. (1981) Electric fields of the brain: the neurophysics of EEG, Oxford University Press, New York, NY.
Rodríguez, E., George, N., Lachaux, J. P., Martinerie, J., Renault, B., and Varela, F. J. (1999) Perception's shadow: long-distance synchronization of human brain activity. Nature 397, 430–3.
Shaw, J. C., Brooks, S., Colter, N., and O'Connor, K. P. (1979) in Hemisphere asymmetries of function in psychopathology, Gruzelier and Flor-Henry, eds., Elsevier/North-Holland Biomedical, Amsterdam, pp. 257–284.
Spencer, K. M., Nestor, P. G., Niznikiewicz, M. A., Salisbury, D. F., Shenton, M. E., and McCarley, R. W. (2003) Abnormal neural synchrony in schizophrenia. J. Neurosci. 23, 7407–7411.
Varela, F. J., Lachaux, J. P., Rodriguez, E., and Martinerie, J. (2001) The brainweb: phase synchronization and large-scale integration. Nat. Rev. Neurosci. 2, 229–239.
Zaveri, H. P., Duckrow, R. B., and Spencer, S. S. (2000) The effect of a scalp reference signal on coherence measurements of intracranial electroencephalograms. Clin. Neurophysiol. 111, 1293–1299.

Appendix

To deduce Eq. 9 from 6, we use two properties of the inverse trigonometric function $\tan^{-1}$

$$\tan^{-1}(x)+\tan^{-1}(y)=\tan^{-1}\left(\frac{x+y}{1-xy}\right) \quad \text{(A1)}$$

and

$$\tan^{-1}(x)=-\tan^{-1}(-x) \quad \text{(A2)}$$

Combining these two equations we obtain

$$\tan^{-1}(x)-\tan^{-1}(y)=\tan^{-1}\left(\frac{x-y}{1+xy}\right) \quad \text{(A3)}$$

substituting $x=s_{nH}/s_n$ and $y=s_{mH}/s_m$ in (A3) and rearranging terms inside the parentheses as indicated above we arrive at Eq. 9

$$\tan^{-1}\left(\frac{\dfrac{s_{nH}}{s_n}-\dfrac{s_{mH}}{s_m}}{1+\dfrac{s_{nH}}{s_n}\dfrac{s_{mH}}{s_m}}\right)=\tan^{-1}\left(\frac{\dfrac{s_m s_{nH}-s_n s_{mH}}{s_n s_m}}{\dfrac{s_n s_m+s_{nH}s_{mH}}{s_n s_m}}\right)$$

$$=\tan^{-1}\left(\frac{s_m s_{nH}-s_n s_{mH}}{s_n s_m+s_{nH}s_{mH}}\right)$$