Assessment of the pharmacological effects of alprazolam on electroencephalography using connectivity indexes not affected by volume conduction

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Introduction

Electroencephalographic analysis techniques have become a very useful tool to assess brain activity and interactions between cerebral regions, that is, the so-called cerebral connectivity analysis. The effects of some drugs have, so far, been studied using spectral analysis and, to a lesser extent, some linear and nonlinear connectivity techniques. New indexes have recently been designed based on assumptions that make them more robust against volume conduction effects that could yield to spurious connectivity results. These new indexes such as the imaginary coherence (IC) [Nolte et al., 2004], the phase-lag index (PLI) [Stam et al., 2007] and the weighted phase-lag index (WPLI) [Vinck et al., 2011] have proven very useful in several fields, for example in characterizing electroencephalographic (EEG) and magnetoencephalographic (MEG) activity of Alzheimer's Disease patients compared to healthy controls.

However, these techniques have not been applied to study the effect of drugs on the brain. The main purpose of the current work was to assess the suitability and effectiveness of these innovative indexes to study the brain connectivity under psychoactive drug treatment, and concretely, the effects of a single dose of alprazolam, a short-acting drug of the benzodiazepine family.

Alprazolam is extensively prescribed for the treatment of anxiety and panic disorders, and peak plasma concentrations are obtained between 0.5 and 2 hours after intake [Greenblat and Wright, 1993]. Being a benzodiazepines, alprazolam induces an enhancement of the inhibitory pathways through their activity on the $GABA_A$ receptor complex, favouring the entrance to chloride ions into the neurons [Haefely, 1990]. Due to the enhancement of the inhibitory pathways a weakening or even an impairment of functional connectivity could be hypothesized.

Database

A single oral dose of alprazolam 1mg was administered to 9 volunteers (aged between 20 and 32 years, mean age 23 years) in a double-blind randomized crossover placebo-controlled design. Volunteers were reported to be in good health and were not allowed to take any psychoactive drugs, caffeine, alcohol and tobacco in the two weeks before the experimental sessions, nor during the one-week washout period between sessions. The study was conducted in accordance with the Declaration of Helsinki and subsequent revisions concerning experimentation in humans, and was approved by the Hospital Ethics Committee and the Spanish Ministry of Health.

EEG signals were recorded for 3 minutes several times: before drug intake, and 30 minutes, 45 minutes, 1 hour, 1.5 hours, 2.5 hours, and 4 hours after the administration. Vertical and horizontal electrooculograms and 19 EEG channels (Fp1/2, F7/8, F3/4, Fz T3/4, C3/4, Cz, T5/6, P3/4, Pz, and O1/2, following the international 10/20 system) were acquired, referenced to averaged mastoid electrodes (A1 and A2). Gold plated standard EEG electrodes were used, keeping impedances below 5 kΩ. Signals were analogically band-pass filtered (0.1 to 45 Hz), and recorded at a sampling frequency of 100 Hz.

Methodology

All EEG recordings underwent ocular artefact reduction following the procedures described by Romero et

al. [2008]. Given that the main effects of alprazolam on cerebral rhythms focus on alpha1 (7.5 to 10.5 Hz) and beta bands (13 to 35 Hz) [Barbanoj et al. 2007], recordings were subsequently filtered using an inverse Chebyshev filter to obtain their corresponding signals. Attenuation in the passband and the stopbands was set to 1 and 20 dB respectively, and forward and reverse filtering was applied to ensure zero-phase distortion.

The considered connectivity measures, namely the phase-lag index (PLI), the weighted phase-lag index (WPLI), and the imaginary coherence (IC), were proposed initially as tools able to quantify the amount of synchronization between pairs of signals without taking into account spurious connectivity due to instantaneous propagation, which could be caused by volume conduction.

The PLI is based on the concept of phase synchronization, assuming that when two signals are coupled, their phase difference is constant or almost constant for a certain amount of time. This synchronization can be calculated by obtaining the phases of EEG signals via the Hilbert transform and calculating the instantaneous difference over time. If there is synchronization between signals, the distribution of these phase differences should be centred on a relevant value. The PLI quantifies the asymmetry of the phase distribution in a scale from 0 to 1, ensuring that a null value is obtained when phase differences concentrate around 0 and π values, which correspond to instantaneous propagation [Stam et al., 2007].

WPLI represents an improvement over PLI because both the sensitivity to noise and the capacity to detect changes in phase synchronization are hindered by the discontinuity of PLI [Vinck et al., 2011]. In WPLI, the contribution of the observed phase differences is weighted by additionally accounting for the magnitude of the phase differences, reducing the probability of detecting connectivity in the case of volume conducted noise sources.

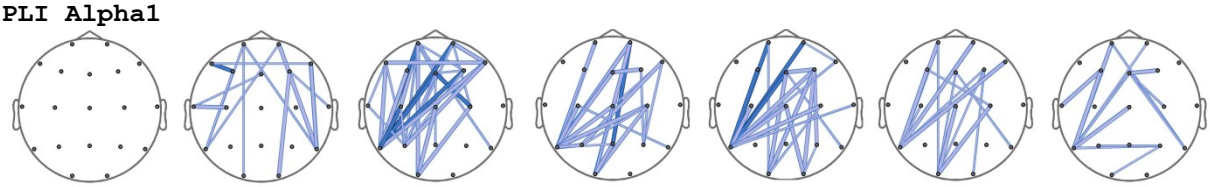
The third considered measure, the IC, is closely related to the standard magnitude squared coherence, but its definition makes it inconsistent with non-interacting sources and that is why it reflects true interaction in contrast to volume conducted self-interaction [Nolte et al., 2004].

Once these indexes were calculated, the pharmacological effects were assessed by means of statistical parametric maps of the head, representing significant differences in connectivity between placebo and drug situations as lines connecting the corresponding scalp locations. Non-parametric Wilcoxon signed-rank tests were used, and an omnibus threshold based on the binomial theorem [Cross-Chaffin, 1982; Alonso et al. 2010] was used to detect significant maps taking into account multiple comparisons.

Results

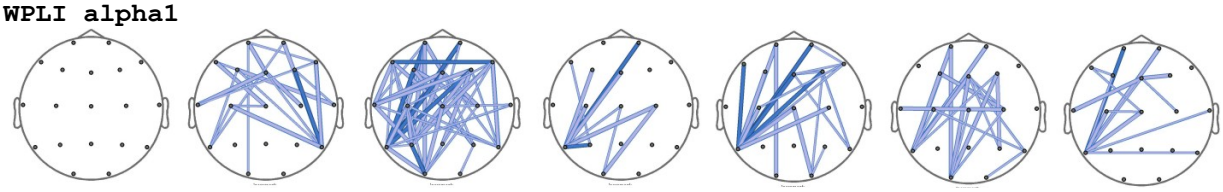
As shown in the following figures, significant changes were found in the alpha1 band (PLI, WPLI, and IC; significant maps are underlined), but not in beta band (maps shown do not reach the minimum threshold stablished by the omnibus test). The obtained changes fall within the expected time-window of peak plasma concentrations.

PLI Alpha1



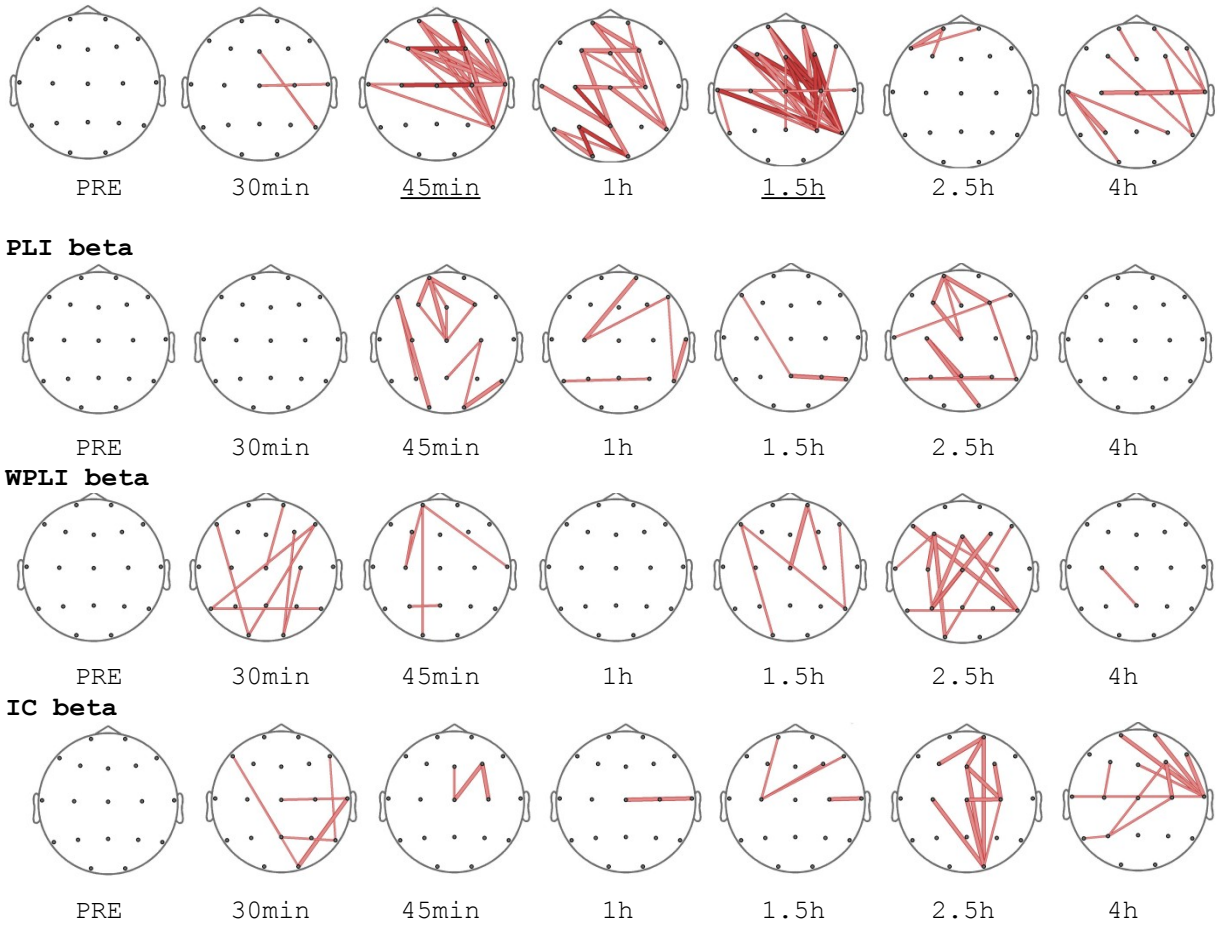
PRE 30min <u>45min</u> 1h 1.5h 2.5h 4h

WPLI alpha1



PRE 30min <u>45min</u> 1h 1.5h 2.5h 4h

IC alpha1



The following table shows the Pearson correlation coefficients obtained when comparing the time course of the values of each connectivity measure with drug plasma concentrations:

	PLI	WPLI	IC
Alpha 1	0.916	0.853	0.957
Beta	0.870	0.792	0.791

Although measures in the beta band cannot elicit significant differences caused by the pharmacological effects, correlation coefficients are high and show that these measures are able to follow these effects.

Conclusions

The suitability of three different connectivity measures, not affected by volume-conducted propagation, for the detection of alprazolam-induced effects on the brain was assessed in this work. Analysis was focused on the previously known bands of interest, alpha1 and beta. The results of the three indexes (the phase-lag index, the weighted phase-lag index, and the imaginary coherence) in these bands match with the pharmacological effect measured by blood levels, but only the alpha1 band shows significant changes associated with drug intake. In this sense, previous studies have successfully extracted such information from spectral and nonlinear connectivity measures that might be affected by volume conduction [Alonso et al., 2010; Alonso et al., 2011]. The special design of the experiment, comparing placebo and drug conditions and correcting by the baseline instant, reduces the effects of spurious connectivity and possibly favours the obtention of results using such measures in contrast to the new indexes studied in the current work.

PLI and WPLI indicate a reduction of phase synchronization due to alprazolam administration, whereas IC shows an increase of interaction. However, the absolute value of the imaginary part of the coherence is

not a true measure of coupling since it depends on the strength of such coupling as well as on the magnitude of the phase difference, so it is essentially quantifying different relationships between signals induced by the drug.

The fact that the considered indexes were not able to find significant differences in the beta band might indicate that phase-coupling changes induced by the drug are weak or too subtle to be detected, given that all measures are corrected by a baseline recording. This might discourage their use in psychopharmacological studies when assessing low doses, mild effects, or when working with a reduced number of participants. However, correlations with plasma concentrations remained high, indicating that PLI, WPLI and IC should not be totally discarded as means of evaluating pharmacological effects on the brain via EEG recordings.

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