Influence of Ocular Filtering in EEG Data on the Assessment of Drug-Induced Effects on the Brain

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Abstract: Ocular artifacts in EEG signals affect the interpretation of clinical study results. The aim of this study was to assess the influence of automatic ocular filtering procedures in the conclusions drawn from a pharmaco-EEG trial. Regression analysis, gold standard, and blind source separation (BSS), Second Order Blind Identification algorithm, ocular filtering procedures were compared using time, frequency, topographic and tomographic brain mapping approaches and pharmacokinetic-pharmacodynamic (PK-PD) relationships. Data consisted of EEGs obtained from 20 volunteers who received single oral doses of haloperidol 3 mg, risperidone 1 mg, olanzapine 5 mg and placebo in a randomized cross-over double-blind design. Although the BSS-based technique preserved brain activity more than regression analysis in anterior leads, in general, topographic significance probability maps globally showed similar results with both methods for most spectral variables. However, different results were obtained when using whole multi-channel information for studying drug effects in the brain: (i) higher correlations between PK and PD time courses showing that BSS allowed estimation of spectral variables more accurately related to drug effects and (ii) larger and more symmetric drug related tomographic LOR-ETA maps showing that BSS led to results that were more neurophysiopharmacologically sound. Definitely, the BSS-based procedure is an effective and efficient preprocessing method to remove ocular artifacts from EEG data. The selection of the ocular filtering procedure could determine different results whose impact depends on the evaluating tool applied to analyze the pharmaco-EEG data. *Hum Brain Mapp* 30:1470–1480, 2009. © 2008 Wiley-Liss, Inc.

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INTRODUCTION

Quantitative analysis of electroencephalographic (EEG) signals is a very useful and practical objective measure in clinical neuropsychopharmacology to evaluate drug bioavailability in the human brain [Saletu et al., 1987]. In pharmaco-EEG studies, drug activity is established by means of EEG changes between pre- and post-medication conditions. These EEG effects are frequently quantified by the calculation of spectral variables in different frequency bands of clinical interest: delta, theta, alpha, and beta. In addition, time courses of these spectral EEG parameters in

combination with drug plasma concentrations allow the estimation of pharmacokinetic-pharmacodynamic (PK-PD) models of drug action to suggest appropriate dose levels and administration intervals [Barbanoj et al., 2002]. Moreover, new applied techniques using multi-channel recordings such as topographic EEG brain mapping and the neuroimaging method known as low-resolution electromagnetic tomography (LORETA) have recently provided more accurate information than individual channel analysis about pharmacodynamics in the brain [Saletu et al., 2006]. Several studies have used LORETA to identify anatomical brain areas predominantly involved in neuropsychopharmacological action [Frei et al., 2001; Riba et al., 2004; Babiloni et al., 2006].

However, some noncerebral interference such as ocular activity also appears in the EEG recordings. Ocular potentials are generated from the electric dipole composed of the cornea and retina. When the ocular globe rotates on its axis, it generates a current field with large amplitude which can be recorded by electrodes at scalp sites. These ocular artifacts must be detected and removed because they can lead to false results and erroneous clinical conclusions. In general, artifact reduction is more desirable than artifact rejection, since no information is lost, and this is especially important when limited data is available.

There are several methods to remove ocular activity from EEG data. The most common approach, considered the gold standard method, is based on regression analysis in time or frequency domains. It estimates factors which model the propagation of ocular activity from electrooculographic (EOG) signals to single EEG channels. Thus, correction based on regression methods involves subtraction of a portion of EOG signals from each EEG channel. The main drawback is that regression-based approaches do not take into account the bidirectional contamination between ocular and cerebral activities in EOG and EEG signals. In other words, regression methods consider that EOG electrodes acquire pure eye activity; however, both EEG and EOG signals record a mixture of ocular and cerebral activities. Therefore, whenever regression-based removal is performed, relevant cerebral information contained in EOG signals is also cancelled in the corrected EEG data.

To solve this drawback, other approaches based on blind source separation (BSS) have shown to be very effective tools for eliminating ocular artifacts from event-related potentials [Jung et al., 2000] and from spontaneous EEG signals [Vigario, 1997]. BSS procedures consider that ocular and cerebral generators are separate anatomical and physiological processes, that is, their related activities must be independent. These methods decompose the EOG and EEG data into source components, identify those that are artifact related, and reconstruct the EEG signals without them. Several algorithms can be used to solve the BSS problem, all of them based on the assumption that sources should be statistically independent [Hyvärinen et al., 2001]. Spatiotemporal decorrelation procedures based on second-order statistics (SOS) have shown the best perform-

ance for eye movement artifact correction in simulated EEG and EOG recordings [Kierkels et al., 2006; Romero et al., 2008]. Although the performance of these different ocular correction techniques has been evaluated on real EEG signals, the true influence of these techniques on the conclusions reached in clinical EEG studies has been not yet analyzed.

The aim of this work was to assess the impact of ocular filtering in evaluating drug-induced effects on the brain using time, frequency, topographic and tomographic brain mapping approaches as well as PK-PD relationships. We evaluated one typical (haloperidol) and two atypical (olanzapine and risperidone) antipsychotic agents widely used in the treatment of schizophrenia and other psychotic disorders [Tandon, 1998]. The drugs were administered to healthy volunteers in highly controlled and standardized laboratory conditions.

MATERIALS AND METHODS

Study Design

Twenty volunteers of either gender (10 males and 10 females) aged between 20 and 32 years (mean age: 23.75) were included in the study. Volunteers were in good physical health, confirmed by medical history, laboratory tests, ECG and urinalysis, and psychological health (Structured Clinical Interview for DSM-IV). Volunteers were requested to abstain from any medications or illicit drug use in the 2 weeks prior to the experimental sessions and until the completion of the study. Volunteers also abstained from alcohol, tobacco and caffeinated drinks 24 h prior to each experimental day. In a double-blind randomized fashion, each volunteer received either a single oral dose of placebo, olanzapine 5 mg, risperidone 1 mg or haloperidol 3 mg in four experimental sessions at least 1 week apart. Although these dosages were lower than clinical doses at low effective therapeutic range (olanzapine 10 mg/day, risperidone 2 mg/day and haloperidol 5 mg/day; [Miyamoto et al., 2002]), they can be considered equipotent from a pharmacodynamic point of view as they were administered to healthy volunteers.

On each experimental day, drugs were orally administered in the morning (8.00 h) under fasting conditions. Serial venous blood samples were taken at predefined times (from +1 h to +10 h in 1 h intervals). Blood samples were heparinized and centrifuged. Plasma levels of haloperidol, risperidone and olanzapine were measured by a validated liquid chromatography tandem mass spectrometry method.

Signal Acquisition

EEG recordings were assessed from 19 electrodes placed on the scalp according to the international 10/20 system on the following locations: Fp1, Fp2, F7, F3, Fz, F4, F8, T3, C3, Cz, C4, T4, T5, P3, Pz, P4, T6, O1 and O2, referenced

to averaged mastoids. Additionally, vertical and horizontal EOG (VEOG and HEOG, respectively) signals were recorded. VEOG was obtained from mid-forehead (2.5 cm above the pupil) and from the average of one electrode below the left eye and another below the right eye (2.5 cm below the pupil). The HEOG signal was acquired from the outer canthi. EEG and EOG signals were recorded using high-pass (0.3Hz) and low-pass filters (45 Hz), with a sampling frequency of 100 Hz, by means of Neuroscan Synamps amplifiers.

Vigilance controlled EEG for 3 min with eyes closed was recorded at baseline, +1, +2, +3, +4, +5, +6, +7, +8, +9, +10, +11 and +12 hours after drug administration. The experimental sessions were undertaken in a quiet room with the volunteers seated in a reclining chair. The experimenter remained outside the room during the vigilance-controlled recordings, and attempted to keep the volunteers alert by acoustic stimulation as soon as drowsiness patterns appeared in EEG recordings.

Signal Preprocessing

A two-step artifact processing procedure was used. The first stage consisted of an ocular artifact reduction process. An automatic artifact (saturation, movement, drowsiness patterns or not really clean EEG data by the first stage) rejection process based on time and frequency domain approaches was then implemented in a second stage as described by Anderer et al. [1992]. Regarding the ocular artifact reduction step, two automatic approaches, based on regression and BSS, were applied for evaluation in further comparisons.

Regression Analysis

Multiple regression analysis assumes that recorded EEG signals (EEG_{raw}) are a linear and time-invariant superposition of different sources. Then, corrected EEG signals (EEG_{corr}) can be calculated by subtracting a fraction of the VEOG and HEOG signals from each EEG channel:

$$EEG_{corr}(t) = EEG_{raw}(t) - \alpha \cdot VEOG(t) - \beta \cdot HEOG(t) \quad (1)$$

where α and β are vectors that represent the propagation factors of the VEOG and HEOG signals, respectively, to the EEG channel. The Eq. (1) was applied to each EEG channel with its corresponding factors α and β . These propagation factors were estimated by regression analysis using only samples of data with high EOG activity [Semlitsch et al., 1986; Anderer et al., 1992].

For the regression procedure, the propagation factors only depended on the subject and the electrode position on the scalp. Therefore, these factors were calculated for each subject using all the data available from the same session (12 recordings of 180 seconds).

BSS Procedure

BSS is a statistical signal processing technique whose goal is to recover source signals from several observed linear mixtures. One way to formulate the BSS problem is to consider the parametric estimation of the following generative model for the data:

$$x = A \cdot s \quad (2)$$

where x is a matrix composed of n row vectors (raw EOG and EEG signals recorded at different electrodes), s is a matrix composed of m row vectors (source signals), and A is an $n \times m$ mixing matrix which must be estimated. In other words, there are some mixtures x_i ($1 \leq i \leq n$) of some original source signals s_j ($1 \leq j \leq m$). The objective is to estimate the original sources without the aid information about the sources or the mixing process:

$$\hat{s} = W \cdot x \quad (3)$$

where rows of the output $\hat{s}$ are the estimated source signals, and the columns of the inverse matrix W^{-1} (estimated mixing matrix A) provide the projection strengths of the respective sources onto the scalp electrodes. Scalp topographies of these projection strengths related to sources allow us to examine the evidence concerning their biological origin and to determine whether a source signal is related to ocular or cerebral activity. In this study, automatic artifact identification was based on frequency and scalp topography aspects of the sources and was previously described in Romero et al. [2004]. After detecting sources related to ocular interference, corrected EEG signals can be reconstructed only from the remaining source components by zeroing out the corresponding rows of the matrix $\hat{s}$ related to eye activity.

There are several estimation principles to solve the BSS problem. These can be divided into SOS-based techniques and ICA (Independent Component Analysis) algorithms based on higher-order statistics. Generally, ICA is essential to solve the BSS problem if sources are assumed mutually independent. However, as EEG and EOG data have temporal structure, less restrictive conditions than statistical independence are often sufficient to estimate the mixing matrix and sources. Thus, SOS techniques assume the hypothesis that sources are only uncorrelated, which is a weak form of statistical independence. Indeed, SOS approaches which are more simple and faster to compute have shown to be more effective than ICA algorithms for removing ocular artifacts from EEG recordings [Ting et al., 2006]. In this article, the selected SOS algorithm was SOBI (Second Order Blind Identification), which uses the time structure provided by the sources to improve the estimation of the model [Belouchrani et al., 1997]. This algorithm has shown the best performance on simulated spontaneous EOG and EEG signals [Kierkels et al., 2006; Romero et al., 2008].

BSS was applied using epoch durations of 90 seconds. This duration was selected in a previous study by means

of an analysis with simulated EEG data [Romero et al., 2008]. This analysis showed that similar percentage errors between sources and corrected EEG signals were obtained for SOBI algorithm in all different epoch durations longer than 5 seconds but they were minimum at 90 seconds.

EEG Processing and LORETA Analysis

After computing the two-step artifact preprocessing procedure, spectral analysis was performed for all EEG channels. Mean percentages of artifact-free 5-s epochs were $65.8 \pm 16.2\%$ and $74.1 \pm 14.0\%$ ($P < 0.0001$) for regression- and BSS-based ocular reduction techniques, respectively. Additionally, the epochs considered after applying regression analysis were also contained in the free-artifact epochs by BSS method. Power spectral density (PSD) functions were calculated from artifact-free 5-s epochs by means of a periodogram using a Hanning window. Spectral density curves for all artifact-free EEG epochs were averaged for a particular experimental situation (treatment X at time Y). These mean PSD functions were quantified into 29 variables: total power (0.5–35 Hz); absolute and relative power of the following frequency bands: delta (0.5–3.5 Hz), theta (3.5–7.5 Hz), alpha1 (7.5–10.5 Hz), alpha2 (10.5–13 Hz), beta1 (13–16 Hz), beta2 (16–20 Hz), beta3 (20–25 Hz), beta4 (25–30 Hz), beta5 (30–35 Hz), combined δ -theta, alpha and beta; and the centre-of-gravity frequency (centroid) of the combined δ -theta, alpha and beta bands as well as of the total activity.

Statistical analysis of EEG recordings was performed following the IPEG (International Pharmaco-EEG Group) guidelines for statistical design and analysis of pharmacodynamic trials [Ferber et al., 1999]. In short, paired *t*-tests were carried out at all observation times, locations and variables to evaluate changes and inter-drug differences in detail at different hours post-administration. All results obtained for a specific time were referenced to the baseline situation before drug intake. According to the experimental design used, pharmacologically sound patterns of *P*-values < 0.05 were those showing spatial clustering and time courses. These results were displayed as topographic significance probability maps. In addition, time courses were also used to estimate the PK-PD relationships of the drugs under study [Barbanoj et al., 2006].

Brain regions associated with EEG effects in antipsychotic-treated volunteers were identified by means of LORETA. The LORETA technique estimates the three-dimensional intracerebral current density distribution from the voltage values recorded at the scalp electrodes [Pascual-Marqui et al., 1994]. The LORETA version (sLORETA) employed in this article implements a three-shell spherical head model used in Talairach's Human Brain Atlas that was digitized at the Brain Imaging Center of the Montreal Neurologic Institute [Talairach and Tournoux, 1988]. Only the artifact-free epochs after the two-step artifact processing procedure were used for computing the LORETA functional images. The LORETA solution was restricted to the

cortical gray matter and hippocampus based on the Talairach atlas. The final solution space consisted of 6,239 voxels with a spatial resolution of 0.125 cm^3 [Pascual-Marqui, 2002]. LORETA images represent the power (i.e., squared magnitude of computed intracerebral current density) in each of the 6,239 voxels.

In a first step, current density values were estimated based on the EEG cross-spectral matrix and then squared for each voxel and frequency band. To evaluate differences between drugs and placebo, paired-sample *t*-tests were computed for log-transformed baseline-corrected LORETA powers at each voxel and each frequency band for the different time points. To correct for multiple comparisons, a non-parametric single-threshold test was applied on the basis of the theory for randomization and permutation tests developed by Holmes et al. [1996]. The omnibus null hypothesis of no activation anywhere in the brain was rejected if at least one *t*-value was above the critical threshold for $P = 0.05$ determined by 5,000 randomizations. On the basis of the Structure-Probability Map Atlas [Lancaster et al., 1997], the number of significant voxels was computed for each hemisphere and for each anatomical lobe: frontal, parietal, occipital, temporal, limbic, and sublobar.

RESULTS

Time Domain

The effects of the proposed ocular correction procedures in time domain on different EEG channels can be observed in Figure 1. As propagation factors are very high (above 0.85) on anterior regions (frontopolar), the regression-based ocular correction method removed some cerebral activity at these sites that was also recorded in EOG channels due to bidirectional contamination. By visual inspection, SOBI algorithm produced more reliable ocular artifact-free EEG signals in anterior placed electrodes. However, no apparent visual differences between ocular correction techniques were observed for central and more posterior EEG scalp locations.

Frequency Domain

Figure 2 depicts the influence of ocular filtering in PDS functions as they are the means by which variables assessing drug-induced effects on EEG are calculated. It shows an example of average PSD functions obtained from frontopolar, frontal and posterior electrodes at baseline, and also at 4 h after olanzapine administration when the maximum effect is expected. Graphs in this figure depict data before and after applying both ocular correction methods. The impact of the drug effects and of the ocular removal method can be observed. The regression-based technique not only reduced ocular-related frequency components but also removed interesting cerebral activity shown by the decrease of alpha peak with respect to raw EEG signals. This reduction appeared in anteriorly-placed electrodes:

Figure 1.

(a) Five-second epoch of raw EEG signals containing a prominent slow eye movement artifact. As an example, only some EEG channels corresponding to left hemisphere are shown. (b) and (c) Corrected EEG signals obtained after applying automatic ocular removal procedures based on regression analysis and Blind Source Separation (BSS), respectively.

frontal and mainly frontopolar. This effect for a specific EEG channel was observed in all recording times: not only after drug administration but also at baseline. In any case, the reduction of the fast EEG activity (alpha and beta) by regression-based ocular removal method can affect the evaluation of the drug effect in these frequency bands.

Spectral EEG Variables

Topographic brain maps comparing drug and placebo-induced changes based on *t*-tests were computed for each of the 29 variables calculated for regression and BSS ocular removal approaches. Significance probability maps between drug-induced and placebo-induced changes in some target variables at 4 h after administration are depicted in Figure 3a,b using regression and BSS, respectively, for ocular reduction. In general, the results were similar with both approaches for central and posterior electrodes. However, differences appeared between regression and BSS-based methods for anterior EEG channels in topographic maps. Moreover, results obtained using the BSS method in the anterior EEG channels were generally more in line with those observed at central and posterior electrodes. That is, alterations found on central and posterior sites were also obtained at frontal sites using the BSS approach: for example, decreases for haloperidol and olanzapine in combined δ -theta centroid; increases for risperidone and olanzapine in absolute delta and combined δ -

theta activity; and decreases for olanzapine in absolute alpha power. These results were not found using the regression approach. It is difficult to account for the fact that a drug shows differences in all EEG leads except frontopolar and lateral-frontal channels where the interference of ocular activity was also maximum.

Two-way ANOVAs were performed to quantitatively evaluate the differences between the two ocular removal methods. The main factors were (i) drug effects (placebo vs drug) and (ii) ocular filtering effects (regression vs BSS). The interaction between these two factors (Fig. 3c) shows whether the effects of the former depended or not on the latter. Two-way ANOVA significance probability maps revealed that the ocular-filtering factor significantly altered drug-induced effects, especially in anterior-placed electrodes. In other words, drug-induced increases (both absolute δ -theta and alpha powers for risperidone; and absolute and relative delta powers for olanzapine) or decreases (combined δ -theta centroid for haloperidol; and both absolute alpha power and combined δ -theta centroid for olanzapine) were higher at the anterior scalp regions when the BSS-based ocular removal approach was applied.

Spectral EEG parameters have been often used to relate PK-PD effects of drugs acting on the central nervous system (CNS) [Ebert et al., 2001; Barbanoj et al., 2002]. The combination of drug plasma concentrations and EEG spectral variables allows PK-PD modeling to be an adequate tool for estimating the time dynamics of drug effects on

Figure 2.

Average PSD functions obtained at baseline and at maximum peak effect of olanzapine (4 h after administration). Frontopolar depicts average values of Fp1 and Fp2 channels; frontal represents average values of F7, F3, Fz, F4 and F8 channels; and posterior indicates

average values of P3, Pz, P4, O1 and O2 channels. Different colors are used to distinguish between PSD functions obtained from recorded or raw EEG signals (black) and from ocular corrected EEG data using regression analysis (blue) or BSS (red).

Figure 3.

Significance probability maps showing differences between drug-induced (haloperidol, risperidone and olanzapine) and placebo-induced changes in absolute δ -theta, delta and alpha powers, and in combined δ -theta centroid at 4 h after administration. Maps are calculated after using regression-based (a) or BSS-based (b) ocular artifact removal approaches. (c) depicts maps based on the interaction

between factors of two-way ANOVAs [drug effects (placebo vs. drug) and ocular filtering effects (regression vs. BSS)]. Electrode positions are indicated by black dots. Seven-color scale represents statistical differences based on p-values. Increases and decreases are depicted as hot and cold colors, respectively.

TABLE I. Absolute values of normalized cross correlation between average concentration-time curve (risperidone and olanzapine) and average effect-time curve of spectral EEG variables (absolute delta and alpha, respectively) obtained in anterior, central and posterior areas after applying regression and Blind Source Separation (BSS) based ocular filtering procedures

Area	Risperidone absolute delta			Olanzapine absolute alpha		
	Regression	BSS	<i>P</i> -value	Regression	BSS	<i>P</i> -value
Anterior	0.72	0.95	0.013	0.83	0.92	0.026
Central	0.89	0.92	0.892	0.90	0.95	0.315
Posterior	0.93	0.94	0.858	0.94	0.95	0.197

Statistical differences (*P*-values) between regression and BSS correlations were obtained by using paired *t*-tests on individual correlations (*n* = 20). Anterior data were obtained by averaging Fp1, Fp2, F7, F3, Fz, F4 and F8 electrodes; central by averaging T3, C3, Cz, C4 and T4; and posterior data by averaging T5, P3, Pz, P4, T6, O1 and O2.

the CNS, including times of onset or peak, duration of drug effects and quantification of dose relations. For most spectral variables, a similar time courses of EEG effects was obtained at central and posterior electrodes for both ocular reduction methods. However, some differences appeared between the two approaches respect to anterior-placed electrodes. Figure 4 shows, as an example, the concentration-time profiles of risperidone 1 mg (4a) and olanzapine 5 mg (4b), and the effect-time profiles of absolute delta (4a) and alpha (4b) powers obtained for both ocular artifact reduction methods. Spectral values were calculated by averaging anterior electrodes after subtracting the baseline and placebo conditions. Curves obtained after applying the regression-based ocular removal technique appeared to present more variability between time points, especially in absolute delta activity after risperidone intake.

To evaluate the correspondence between concentration-time profiles and effect-time profiles of spectral EEG variables, a normalized cross correlation between these two curves was performed for both ocular filtering procedures. Table I shows these correlation values between concentration-time curves (risperidone and olanzapine) and effect-time profiles (absolute delta and alpha, respectively) obtained for anterior, central and posterior electrodes after applying both ocular filtering methods. Values at central and posterior brain areas were very similar for both methods. However, for risperidone, the effect-time curve of absolute delta power (anterior lead) obtained after the BSS-based ocular removal technique correlated better with the concentration-time curve than that obtained after using the regression-based method (0.95 vs 0.72; *P* = 0.013, paired *t*-test). Moreover, the effect-time profiles of absolute alpha activity (anterior location) obtained using the BSS-based method also correlated better with the olanzapine plasma concentrations curve (0.92 vs 0.83; *P* = 0.026, paired *t*-test). Thus, only the correlation values calculated after applying the BSS-based procedure in anterior leads were similar to those obtained in central and posterior areas after applying either method.

LORETA Data

Omnibus significance tests were performed for all voxels and frequency bands at 4 h (baseline corrected) after drug administration, corresponding to the maximum peak effect. Different results were found in some frequency bands depending on the ocular reduction method used. Statistical overall analysis applied as voxel-by-voxel comparison of olanzapine versus placebo-induced effects 4 h after drug administration revealed statistically significant increases in the delta frequency band and decreases in the alpha band. No significant differences were obtained after haloperidol or risperidone administration at this time point. Figure 5 shows, as an example, 3-D surface-rendered LORETA images as statistical nonparametric maps corresponding to suprathreshold regions found for the alpha frequency band, 4 h after olanzapine administration. These statistical tomographic maps were obtained using both ocular removal techniques. By visual inspection, the BSS-based method seemed to provide more symmetric maps than the regression approach.

In addition, Table II lists the anatomical distribution of these power decreases observed for the alpha frequency band at 4 h after olanzapine administration for both ocular artifact reduction methods. Results indicated that while 1,756 suprathreshold voxels were located for the BSS-based procedure, only 513 were obtained after the regression-based ocular removal technique. Moreover, in the area most related to alpha rhythm (occipital and parietal lobes), the decrease in alpha LORETA power after applying the regression approach seemed more pronounced over the right hemisphere than over the left (379 vs 31 voxels, respectively), whereas for the BSS approach, decreases were less asymmetric (761 vs 506, respectively).

DISCUSSION

Ocular contamination of EEG data is a very important and common problem in clinical EEG studies evaluating

Figure 4.

Pharmacokinetics and pharmacodynamics of (a) risperidone 1 mg and (b) olanzapine 5 mg. Black lines represent observed drug plasma concentration time-profiles in ng/ml. Blue and red lines represent absolute delta (4a) and alpha (4b) powers (μV^2) after applying regression or BSS-based ocular removal methods,

respectively. Spectral values were calculated by averaging anterior electrodes (Fp1, Fp2, F7, F3, Fz, F4 and F8) after subtracting the baseline and placebo conditions. Time is depicted on the x-axis and the concentration and absolute powers are depicted on the y-axis.

Figure 5.

Effects of olanzapine 5 mg on regional cortical electrical activity 4 h after administration ($n = 20$). Images seen from different perspectives show statistical nonparametric maps based on t values of differences between olanzapine-induced and placebo-induced changes in alpha frequency band (7.5–13 Hz) after apply-

ing regression- and BSS-based ocular reduction procedures. Blue indicates significant decreases after Holmes correction ($P < 0.05$) as compared to placebo. Structural anatomy is shown in gray scale (L: left, R: right, A: anterior, P: posterior, S: superior, I: inferior).

TABLE II. Suprathreshold voxels obtained for olanzapine- vs placebo-induced decreases in alpha power (7.5–13 Hz) 4 h after administration (n = 20)

Lobe	Regression						Blind source separation (BSS)					
	Left hemisphere			Right hemisphere			Left hemisphere			Right hemisphere		
	N_{SIG}	N_{TOT}	%	N_{SIG}	N_{TOT}	%	N_{SIG}	N_{TOT}	%	N_{SIG}	N_{TOT}	%
Frontal lobe	0	1026	0	0	1150	0	0	1026	0	0	1150	0
Parietal lobe	0	569	0	39	584	7	231	569	41	362	584	76
Occipital lobe	31	362	9	340	399	85	275	362	76	399	399	100
Temporal lobe	0	576	0	70	584	12	13	576	2	216	584	37
Limbic lobe	0	335	0	33	424	8	70	335	21	169	424	40
Sublobar lobe	0	113	0	0	117	0	0	113	0	22	117	19

Values for both ocular removal approaches are shown. The number of significant voxels (N_{SIG}), the total number of voxels (N_{TOT}) and the percentage of significant voxels (%) for each lobe and hemisphere are given.

drug effects on the brain because PSD functions of ocular and cerebral activities overlap in frequency. Although previous studies have evaluated the performance of several ocular artifact reduction methods on simulated and real EEG signals, none have yet revealed the practical influence of ocular artifact removal in a real clinical situation such as a pharmaco-EEG trial.

In this study, an automatic BSS-based ocular artifact reduction procedure was compared with the “gold standard” method based on regression analysis to evaluate the influence of ocular artifact filtering in pharmaco-EEG trials. To determine the impact of artifact reduction on drug-induced results and conclusions, different approaches were applied: time domain, PSD functions, topographic maps based on spectral target variables comparing drug with placebo-induced alterations, PK-PD time courses, and LORETA tomography, a neuroimaging technique for neurophysiological data.

Results from time domain and PSD functions (see Figures 1 and 2) indicated that the BSS-based method preserved and recovered much more brain activity than regression analysis in anterior-placed electrodes. No visual differences between the two correction techniques were observed for the central and more posterior EEG channels. However, regarding topographic significance probability maps, similar results were obtained for the two artifact removal methods for all spectral EEG variables except some in anterior EEG channels (see Fig. 3). The fact that results were comparable after both ocular artifact removal methods could be due to the within-subject comparison design. Using a design with these characteristics, drug spectral variables are compared to the baseline conditions and to the placebo administration. Interesting brain information is removed because of both, bidirectional contamination phenomena and the regression-based method. As this occurs at baseline and after drug or placebo administration it is not so evident in the graphic display of statistical results, that is in the topographic significance probability maps. In spite of this, some differences between the two methods

were found at anterior sites: results obtained for the BSS-based ocular removal approach were more in line with those observed at central and posterior leads. Thus, taking global information from topographic maps into account, deviations between ocular removal approaches did not provide different pharmacological conclusions concerning drug bioavailability in the brain.

Some multi-channel EEG evaluating techniques, such as PK-PD relationships and LORETA tomography, assess the global information provided rather than analyze the data from each electrode separately.

PK-PD relationships are used to enlarge the scope of inferences that can be derived from a pharmaco-EEG study and include not only the pharmacological properties of the drugs but also the properties of the systems with which the drugs interact [Valle et al., 2002]. One as yet unsolved issue related to PK-PD relationships is which lead to use, a challenge which is currently approached by applying the average from all leads within a target spatial cluster [Barbanoj et al., 2006]. The ocular filtering procedure utilized could play a relevant role in obtaining reliable spectral EEG variable time-profiles. In this study, time curves of spectral EEG variables obtained after applying regression-based ocular artifact reduction technique at anterior sites presented more unaccountable oscillations than those obtained after applying BSS method (see Fig. 4). Moreover, correlations between drug-plasma-concentration-time curves and drug-effect-time curves using these spectral EEG variables from anterior areas were statistically higher when applying BSS-based ocular removal method than when using the regression-based approach. Finally, correlation values for anterior, central and posterior areas obtained after applying BSS ocular reduction were very similar, whereas the values obtained with the regression method at anterior leads were different from the remaining brain areas (see Table I). We consider this indicates that the BSS-based ocular reduction method permits a more accurate estimation of spectral variables related to the drug effect.

Drug topographic maps do not allow spatial interpretation of the results. Scalp distributions are ambiguous and cannot be interpreted directly in terms of local brain electrical generators. In recent years, some computerized methods from dipole analysis to distributed source analysis have been developed to overcome this problem [Michel et al., 2004]. One of them, LORETA, was developed as a source localization method to obtain the most plausible solution of the EEG inverse problem as the smoothest of all possible inverse solutions [Pascual-Marqui et al., 1994]. Several studies have shown that LORETA is a useful tool to localize brain areas involved in the neurophysiological effects of psychoactive drugs [Riba et al., 2004; Babiloni et al., 2006; Saletu et al., 2006]. As LORETA finds the 3-D distribution of the generating electric neuronal activity from measurements of scalp EEG data, changes in any of these EEG signals by applying different ocular reduction techniques can induce different inverse problem solutions for all brain areas. In the present study, we obtained some differences using both ocular removal methods in the LORETA-based analysis. In this case, the reduction of cerebral activity by regression-based technique mostly in frontal regions affected the solution in the brain areas involved in the generation of alpha activity that were located in the parietal and occipital lobes. As an example, LORETA tomographic olanzapine- vs placebo-induced maps of alpha activity after regression-based ocular reduction were more shifted to the right hemisphere in comparison with those after the BSS-based technique (see Fig. 5 and Table II). This asymmetry is not easy to explain from a neurophysiopharmacological point of view.

In conclusion, automatic ocular artifact correction for spontaneous EEG signals by BSS using SOS showed similar and even better performances than the regression-based method when assessing drug effects in the brain. Differences favoring the BSS approach were evidenced when applying pharmacoo-EEG evaluating tools which used information from each electrode to proceed with their analytical procedures. The BSS-based method is an effective and efficient preprocessing tool to remove ocular artifacts from EEG data in pharmacoo-EEG studies to assess drug bioavailability in the brain.

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