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THE INTERPLAY OF LORAZEPAM-INDUCED BRAIN OSCILLATIONS: MICROSTRUCTURAL ELECTROMAGNETIC STUDY

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Abstract

Objective: The effects on cortical rhythms of a single-dose (30 μg/kg) administration of the $GABA_A$ agonist lorazepam were examined in a randomized, double-blind, cross-over, placebo-controlled study with 8 healthy volunteers using simultaneous electroencephalography (EEG) and magnetoencephalography (MEG).

Methods: The oscillations were assessed by means of adaptive classification of short-term spectral patterns.

Results: Lorazepam a) decreased the percentage of EEG/MEG segments with fast-theta, delta-alpha, fast-theta–alpha and alpha activity and increased percentage of EEG/MEG segments with delta, delta–slow-theta, delta-beta, slow-theta and polyrhythmic activity; b) decreased diversity of EEG/MEG signals (in terms of spectral patterns) and increased the general instability of the signal; c) increased stabilization periods of the spectral patterns (reduced brain information processing); d) maintained larger maximum periods of temporal stabilization for delta, slow-theta, delta–slow-theta, delta-beta and polyrhythmic activity (in terms of spectral patterns); e) did not increase power in the independent beta rhythm.

Conclusion: Lorazepam caused significant reorganization of the EEG/MEG microstructure. These results suggest also that adaptive classification analysis of single short-term spectral patterns may provide additional information to conventional spectral analyses.

Keywords: *Adaptive classification, Electroencephalography (EEG), Lorazepam, Magnetoencephalography (MEG), Microstructure, Short-term spectral patterns.*

1. Introduction

Different psychotropic drugs appear to have their own "EEG portraits". Barbiturates induce "barbiturate bursts" in the EEG (Schallek and Schlosser, 1979), whereas benzodiazepines significantly increase power in the slow (1–7 Hz) and fast (13–20 Hz; 21–30 Hz) wavebands while reducing power in the mid-range (8–12 Hz) (Greenblatt et al., 1989; Link et al., 1991; Mandema et al., 1992).

Spectral EEG parameters are usually derived from averaged EEG power spectra, based on extended periods of time and/or broad fixed frequency bands for a specific lead. This has yielded a series of clinically relevant findings. However, the averaging of the EEG signal might not only mask the dynamics of potential effects of drugs on EEG, but also may lead to ambiguous data interpretation (Fingelkurts et al., 2002). At present, almost all methods of quantitative EEG analysis are based on certain implicit assumptions regarding the statistical characteristics of EEG, particularly with respect to the extent of stationarity and Gaussianity of the process. The efficacy of analytic techniques depends upon the degree to which such assumptions are justified by the characteristics of the EEG being analyzed (McEwen and Anderson, 1975). Experiments and analytical work have established several important facts. First, the ongoing EEG is characterized by natural dynamics and piecewise stationary structure (see the reviews Kaplan and Shishkin, 2000; Fingelkurts and Fingelkurts, 2001). Second, the power variability of the main EEG spectral components for successive short (5–10 sec) EEG segments is in the range of 50–100% (Oken and Chiappa, 1988). Third, in terms of the EEG spectral variability, not only the stochastic fluctuations of the EEG parameters, but also a temporal structure of the signal can be observed (see the review, Kaplan, 1998). Moreover, the average spectral characteristics of a broad frequency band predominantly reflect an influence of high-amplitude synchronized segments of the long EEG epochs and the low-amplitude desynchronized ones may be totally obscured (Lazarev, 1998). Fourth, factor analysis of the narrow-band EEG spectra (Herrmann, 1982; Lorig and Schwartz, 1989) have demonstrated that various spectral bands can be grouped in sufficiently complex and dynamic way, which is far from the traditional scheme of linear frequency distribution from delta to gamma-bands. Hence, when examining the average brain electromagnetic responses to drug administration, it is not clear whether the observed effect of the drug is real (not the "virtual" result of averaging procedure), stable and typical for the whole

analyzed signal. For example, it is not clear: (1) whether the change of total power of particular brain oscillations results from a change in the number of their occurrence per minute rather than change of the average oscillations' amplitude, and (2) whether change of total power of particular brain oscillations affects the whole analyzed signal or a small its portion. Thus, regardless of how powerful or statistically significant the different estimations of averaged EEG/MEG effects may be, there might be difficulties in making meaningful interpretations of these if they are not matched to the EEG/MEG piecewise stationary structure (Effern et al., 2000; Laskaris and Ioannides, 2001).

In order to overcome the limitations of conventional spectral analysis based on averaging procedures and to reveal dynamic and temporal characteristics of brain activity the short-term spectral analysis was introduced (Barlow, 1985; Jansen and Cheng, 1988; Hilfiker and Egli, 1992; see also the review Kaplan and Shishkin, 2000). Assuming that the duration of the minimal stationary segment of an EEG is usually no more than 2 s (McEwen and Anderson, 1975; Inouye et al., 1995), it is possible to obtain an entire set of individual short-term spectra of various types in accordance with the number of stationary EEG segments. The parameters of the relative presence of the individual EEG segments of each type and the peculiarities of its alternation in the analyzed EEG may provide additional characteristics of the normal and pathological brain activity (Jansen, 1991; Fingelkurts et al., 2000, 2002, 2003) and possible drug effects on brain dynamics (Kaplan et al., 1996). Current paper presents an application of the proposed approach to the standard clinical problem of testing influence of psychotropic drugs on EEG/MEG. Here, we report on the role of brain oscillations during administration of lorazepam by means of the *adaptive classification analysis* of single short-term spectral patterns (Kaplan et al., 1999; Fingelkurts et al., 2003).

EEG has been used to detect drug effects for decades but studies using MEG are scarce. MEG can be used to measure spontaneous rhythms in humans (Salmelin and Hari, 1994). The unique feature of MEG is the transparency of the skull, scalp and brain tissue to the magnetic fields. An EEG signal, however, is influenced by the head properties. Another difference between EEG and MEG is that the latter measures mostly primary currents oriented tangentially with the surface of the head (in sulci). EEG is sensitive to both radial (gyri) and tangential sources. To our knowledge there have been no previous studies investigating effects of psychotropic drugs on

spontaneous brain activity using MEG. Due to their different sensitivity, the combination of MEG with EEG may provide more comprehensive view about drug effects on brain functioning.

Benzodiazepines, $GABA_A$-agonists that potentiate neural inhibition, constitute a large group of drugs which are widely used in clinical practice (Pohlman et al., 1994; Kennedy and Longnecker, 1996; Swart et al., 1999; Alldredge et al., 2001). Since benzodiazepines are often used for clinical purposes, knowledge of the validated EEG/MEG effects of benzodiazepines, and temporal characteristics of these effects are necessary for the interpretation of the results.

Additionally, benzodiazepines produce effects whose link to clinical efficacy or side effects are not well established. For example, interpretation of increased EEG beta activity is often difficult from different studies (Koelega, 1989), thus making it important a closer investigation of the cause of changes in the power estimates.

Some of these difficulties can be resolved through a more uniform application of the micro-structural signal analysis techniques. The selection of a set of sensitive EEG/MEG indices, which reflect the dynamic behavior of temporal EEG/MEG structure, may enable the prediction of the brain's response to the drug and adds additional value to standard spectral analysis. Therefore, the main objectives of this study were (1) to validate and verify a number of EEG/MEG-effect parameters, (2) to uncover the temporal characteristics of EEG/MEG dynamics, (3) to provide a more complete description of EEG/MEG oscillations and (4) to study the cause of changes in the power of EEG/MEG beta activity after lorazepam administration in humans using an adaptive classification analysis of the individual short-term spectral patterns. We hypothesize that lorazepam would significantly modify the micro-structural organization of EEG/MEG, which is measured as changes in the balance of the number and the duration of EEG/MEG segments of different types (characterized by spectral patterns).

2. Materials and Methods

2.1. Subjects

Eight non-smoking healthy right-handed subjects (4 males, 4 females, with ages from 20 to 29 years) participated the study. They gave informed written consent; institutional ethical committee approval was obtained. Before inclusion, the subjects underwent a medical

examination and laboratory tests of blood to exclude physical or mental health problems. They were also screened for mental problems by SCL-90 (Derogatis et al., 1973; Holi et al., 1998) and were mild drinkers (maximally five drinks/week). The subjects reported having used no medication during the 2 weeks prior to the study. Subjects' weights averaged 65.9 kg (range: 54–76 kg). The subjects were instructed to avoid alcohol for at least 48 h, and caffeine for 12 h prior to the recordings.

2.2. Trial design

All subjects arrived at the laboratory at approximately 7:30 a.m. after an overnight fast. As different food components may differently alter the subject's ongoing brain activity, an overnight fast intended to provided a degree of equality of the initial conditions. Following electrode placement and instruments calibration, the subject was seated in a comfortable chair in the dimmed registration room. To reduce muscle artifacts in the EEG signal, the subject was instructed to assume a comfortable position and to avoid movement. A subject was instructed to look straight (in the case of eyes open) and to avoid unnecessary eye movements. The behavior of the subject was monitored via TV throughout the experiment.

A catheter was placed in the right antecubital vein for drug injection. Subjects underwent either lorazepam (Ativan® 4 mg/ml, Wyeth Lederle) 30-μg/kg or placebo (saline) injection in a randomized, double-blind, placebo-controlled, cross-over design study. The recording was started 5 min after the infusion. All experimental sessions were carried out between 08:00 h and noon; successive sessions were separated by one week. Subjects underwent simultaneous EEG and MEG registration with eyes closed for 5 minutes and eyes open for another 5 minutes, the order of these conditions being counterbalanced across subjects.

Vigilance of the subjects was controlled by the presence of sleep spindles which naturally appear during drowsiness (Rechtschaffen and Kales, 1968) or may be induced by sleep-inducing drugs (Durka and Blinowska, 2001; Durka et al., 2002). Criteria for sleep spindles detection: (1) visual detection: frequency 12-14 Hz; time duration 0.5-2.5 sec., i.e. one should be able to count at least 6 or 7 distinct waves within the half-second period; peak-to-peak amplitude above 15 mkV (Rechtschaffen and Kales, 1968; Zygierewicz et al., 1999); (2) spectral analysis: an increase of

power in the spectral band related to sleep spindles 12-15 Hz and decrease in slow wave activity in 0.75-4 Hz (Zygierewicz et al., 1999; Durka and Blinowska, 2001).

2.3. Data acquisition

All recordings were performed in the magnetically shielded room (Euroshield, Eura, Finland) of the BioMag Laboratory, Helsinki University Central Hospital. Spontaneous brain activity was recorded with a 306-channel MEG and 64-channel EEG data acquisition system (Neuromag Vectorview, Helsinki, Finland) with the frequency band of 0.06 to 86 Hz (sampling rate 300 Hz). The exact location of the subject's head with respect to the marker coils placed on the scalp were determined in relation to three anatomical landmark points (the nasion and both preauricular points) using a 3D-digitizer (Polhemus, Colchester, VT, USA).

EEG was recorded with an electrode cap (Virtanen et al., 1996) and an amplifier (Virtanen et al., 1997) specifically designed and build for simultaneous EEG and MEG measurements. The nose electrode was used as reference. Reasons for not using average reference (AR) need some clarifications. Although the average reference (AR) approach provides a sound theoretical solution to the EEG reference problem, it nevertheless has limitations that stem from the several approximations on which it rests. Most importantly, AR works only if a high spatial electrode density is available and if a large area of the head is covered (Bertrand et al., 1985). These two assumptions pose a serious problem that was investigated in several studies, but no consensus could be obtained with regard to the minimum electrode density and head coverage that is necessary to obtain unambiguous results (see Chung et al., 1996; Srinivasan et al., 1998; Junghöfer et al., 1999). Then, the posterior alpha rhythm appears to be mirrored at the central coronal line (for empirical demonstrations, see Hjorth, 1980; for a computer simulation, see MacGillivray and Sawyers, 1988). Thus, the increased anterior alpha activity as recorded with an AR of scalp electrodes might be interpreted as an artifact of the reference.

The impedance of the recording electrodes was always below 5 kΩ. Vertical and horizontal electro-oculograms were recorded. The locations of the EEG electrodes and the marker coils in relation to the cardinal points on the head were determined with the digitizer.

MEG and EEG epochs containing artifacts due to eye blinks, significant muscle activity, or movements were automatically rejected. Cardiac interference at low frequencies was also found to

be minimal, with no spectral peak detection at the heartbeat frequency of around 1 Hz, or its harmonics. The presence of an adequate signal was determined by visually checking each raw signal on the computer screen after automatic artifact rejection.

2.4. Data processing

The EEG/MEG data were split into 4 distinct groups: lorazepam closed eyes, lorazepam open eyes, placebo closed eyes, placebo open eyes. Data processing was performed separately for each 1-min portion of the signal. For the tools used for data processing, the EEG/MEG signals were resampled at 128 Hz. Special calculations were done prior to the sampling rate downsampling to attain whether aliasing would be significant and/or affect the results. The power spectra graphics clearly showed that the interpolation-downsampling from 300 to 128 Hz should not affect our results. In fact downsampling can slightly reduce/smooth the power spectra, but it does not generate any frequencies itself (also note that only the main peaks of spectral patterns are relevant in our approach). Fifty-nine MEG locations roughly corresponding to standard EEG sites ($O_{1/2}$, Oz, $PO_{3/4}$, $PO_{7/8}$, POz, $P_{1/2}$, $P_{3/4}$, $P_{7/8}$, Pz, CPz, $CP_{1/2}$, $CP_{3/4}$, $TP_{7/8}$, $TP_{9/10}$, $C_{1/2}$, $C_{3/4}$, $C_{5/6}$, Cz, $T_{7/8}$, $FC_{1/2}$, $FC_{3/4}$, $FC_{5/6}$, FCz, $FP_{1/2}$, FPz, $FT_{7/8}$, $FT_{9/10,}$ $F_{1/2}$, $F_{3/4}$, $F_{7/8}$, Fz, $AF_{3/4}$, $AF_{7/8,}$ AFz) were selected. The planar gradiometer signals were analyzed in this study, as they give the largest signal right above the cortical source and thus straightforwardly help to distinguish activity in different brain areas.

Prior to the spectral analysis, the EEG/MEG signals were bandpass-filtered in the 0.5–30-Hz frequency range. This frequency range was chosen because approximately 98% of spectral power lies within these limits (Thatcher, 2001) and the main lorazepam effects on brain oscillations are found in this frequency band (Link et al., 1991). Thereafter, individual power spectra were calculated in the range of 0.5–30 Hz with 0.5-Hz resolution (61 values), using FFT with 2-sec Hanning window shifted by 50 samples (0.39 s) (Fig. 1) for each selected EEG/MEG location. These values revealed the best results in disclosing temporal patterns from the signal (according to a previous study). In the case of MEG, this sliding spectral analysis was performed for each of the two gradiometers ($\partial Bz/\partial x$ and $\partial Bz/\partial y$, where x, y, z refer to a coordinate system local to each sensor separately; z refer to the detection perpendicular to the scalp). The power spectra for the two gradiometers in each location were averaged separately. As a result, the total

number of individual spectral patterns (SP) for each channel of 1-min EEG/MEG recordings was 149 (Fig.1). These SPs formed the multitude of the objects for further classification.

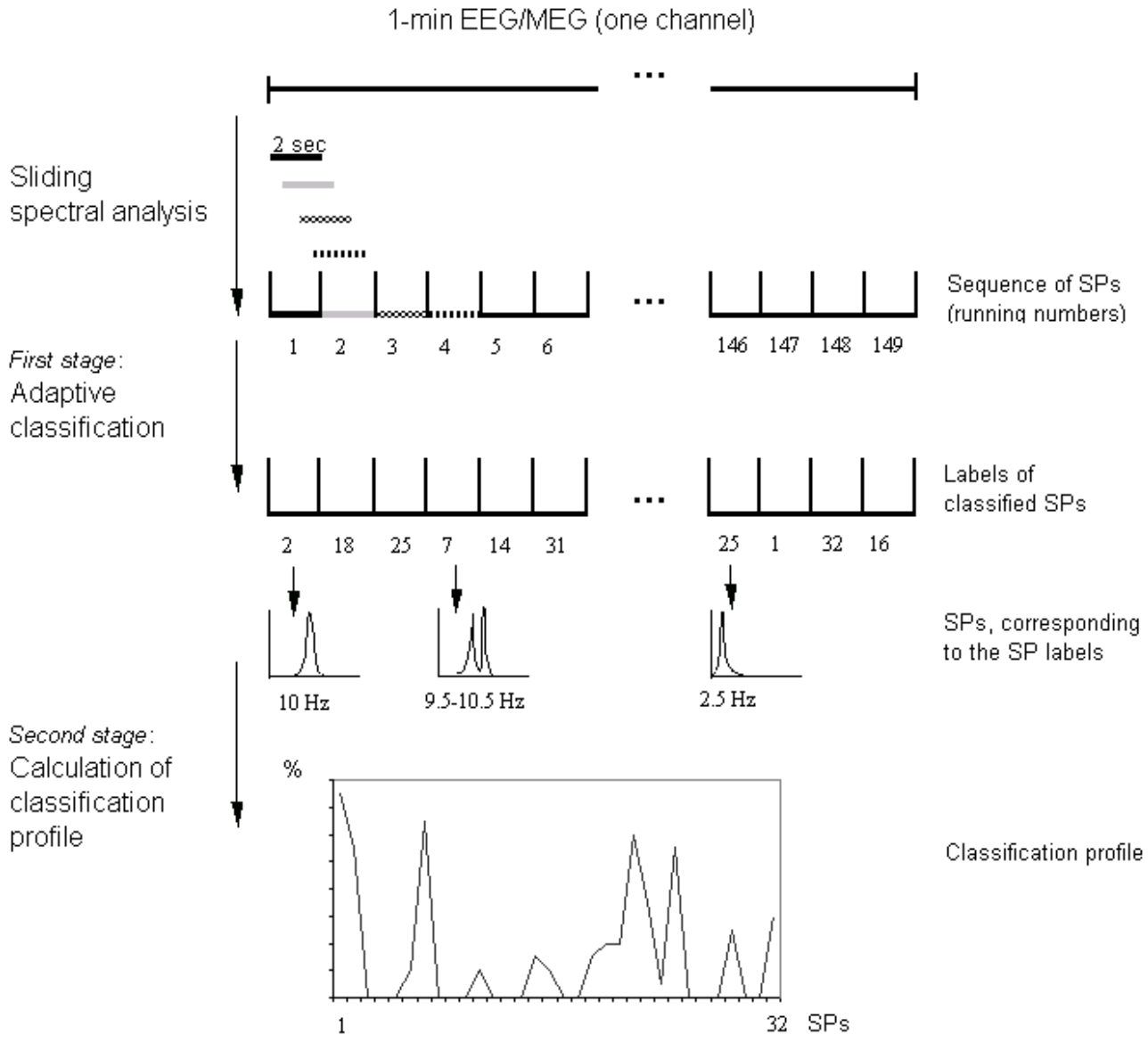

Figure 1. The scheme of the data processing. Sliding spectral analysis, adaptive classification of spectral patterns (SP) and calculation of the classification profiles (CP) were done separately for each subject and each location of 1-min EEG/MEG.

Spectral-pattern (SP) variability for each 1-min signal for each condition was estimated in two stages. At the first stage, sequential single EEG/MEG power spectra were adaptively classified in each EEG/MEG location using the set of standard SPs (Fig. 1). The set of standard SPs (included 32 SPs) was formed using heuristic procedures and Pirson's correlation coefficients (CC) (only those SPs which had minimum mutual correlation, were selected). Basic procedure of adaptive classification was performed in three steps. During the first step, the initial matrix of mutual correlations between standard and current individual SPs of analyzed EEG/MEG was calculated (for each channel separately). On the basis of CC which were obtained at the first step, the current short-term SPs were sorted: all current SPs for which the CCs were equal or exceeded the value 0.71 were attributed to the corresponding standard classes. During the second step, the current SPs which were included in a particular class, were averaged within this class. This procedure was performed for all classes separately for each EEG/MEG channel. On the back of this, the standard spectra were reconstructed but taking into account the peculiarities of spectral description of concrete channel of the particular EEG/MEG. Thereby an "actualization" of the initial standard SP set was performed. In other words, they were converted into so-called actual spectral patterns. This actual SP set was used further for the third step - the final classification of the current SPs. Details of this procedure (SCAN0.1® algorithm, suggested by Prof. Kaplan, Moscow State University (Kaplan et al., 1999)) can be found in Fingelkurts et al., (2003). Adaptive classification technique includes several adequate correction algorithms for considerable reduction of the variance of the single spectral estimations. This justifies the usage of individual short-term SPs and increases the sensitivity of this analytical approach for EEG/MEG dynamics. This SP classification method made it possible to identify up to 100% of individual single spectra in the initial EEG/MEG recordings due to algorithm's adaptivity to local signals. Considering that a single EEG spectrum illustrates the particular integral dynamics of tens and hundreds of thousands of neurons in a given cortical area at a particular point in time (Dumermuth & Molinari, 1987), the SPs within each class can be considered effectively generated by the same dynamics, with the same driving force. Whereas SPs from different classes can be considered to have had different driving forces and therefore have been effectively generated by different dynamics (Manuca and Savit, 1996). In this case, one SP may be considered as single event in EEG/MEG phenomenology from viewpoint of its spectral characteristics. Each SP can be labeled by the index of the class to which it belongs. Thus, a sequence of SP labels that represents the sequence

of EEG/MEG oscillatory states through which the system passes can be obtained. Hence, each EEG/MEG signal was reduced to a sequence of individual classified SPs.

At the second stage, the classification profiles (CP) of spectral patterns for each EEG/MEG location in each subject and for the group of subjects as a whole were calculated (Fig. 1). This index was calculated as the relative number of cases of SP type as a percentage of the total amount of all SPs in any given EEG/MEG location – the histogram of the relative presence of each SP type (Fingelkurts et al., 2003).

CPs were averaged across the five 1-min EEG/MEG signals for each subject separately for each EEG/MEG location and condition. Since results were reproduced for each of the subjects, the data for each condition was averaged across all subjects (separately for each EEG/MEG location). It was expected that these CPs would make it possible to portray (in SP description) lorazepam effects in detail.

In addition, three indices were calculated for each subject separately for each condition and channel of each 1-min EEG/MEG:

a) The percentage of *polyrhythmic/disorganized activity* (PA) – presented by polyrhythmic spectral patterns. A polyrhythmic spectral pattern constitutes a pattern, where peaks occupy majority of the frequencies within the studied range. Such spectral pattern indicates a mixture of activity of small neuronal subpopulations each with its own mode (Tirsch et al., 2000).

b) Index of *non-homogeneity of classification profile* (NHCP) was estimated as a ratio of the number of SP types detected in a given 1-min EEG/MEG to the total number in the standard set (32 standard SPs – 100%). This index indicates how many different SP types participate in CP.

c) Index of *non-stability of classification profile* (NSCP) is a percent value that reflects how the set of distinct SP types changes along the three EEG/MEG sub-segments of 20-sec of a complete 1-min.

$$NSCP = \left(1 - \frac{n1 + n2 + n3}{3(u1 + u2 + u3)}\right) * 100, \quad ui \le ni$$

Where n_i, is the number of distinct SP types found in a 20-sec EEG/MEG segment i; u_i is the number of unique SP types found in segment i and not existing in any other segment. The range of this index is 0–67.

2.5. Statistics

We studied the behavior of each type of spectral patterns separately and did not make any conclusions about differences in classification profiles. In order to reveal statistically significant differences between lorazepam and placebo, Wilcoxon matched pairs test was applied. To control for repeated observations of the same measures a Bonferroni correction was made. $P_{corrected}$ is the value required to keep the number of false positives at $P = 5\%$. Results are reported as average values with standard deviations.

Presented here lorazepam effects were obtained for 8 subjects separately and results were reproduced for 7 of them (notice that it was double-blind experiment). Data for eighth subject was presented separately. Additionally, lorazepam effects were the same for more than 65% of EEG locations. Moreover, the same results were reproduced for MEG. Such consistency and reproducibility of the results testify that the obtained results can't be occasional because "…by definition chance findings do not replicate" (Duffy et al. 1994, p. XI).

Surrogate data were used to control for the neural origin of temporal dynamics of SPs, which is commonly applied as direct probing a signal for a non-random temporal structure (Ivanov et al., 1996). Surrogate signals have identical parameters with the original signals but do not have temporal correlations. Thus, each channel of the actual EEG/MEG was subjected to a randomized mixing of SPs. In such a way, the natural dynamics of SP sequence within each EEG/MEG channel were completely destroyed, but the percentage ratio between different types of SPs remained the same. This modified EEG/MEG was described as "random".

3. Results

By using the adaptive classification method, 100% of individual EEG/MEG SPs were successfully classified. Polyrhythmic spectra characterized 0–11% of EEG and 24–45% of MEG (for different locations).

3.1 General characteristics of lorazepam-induced EEG/MEG changes

Seven of the eight subjects showed an identical lorazepam effect (see below); this effect was similar for EEG and MEG. Since there were no significant differences in the results for these seven subjects, the entire data were averaged across them. Data for the eighth subject (S8) was analyzed separately. The general effect for EEG and MEG averaged across seven subjects and for subject S8 (EEG) is shown in Figure 2. Analysis of the classification profiles (CP) demonstrated that EEG/MEG during placebo was characterized by a larger percentage of alpha- (SP1 (main peak at 8.5 Hz); SP2 (10 Hz); SP3 (11.5 Hz)), fast-theta- (SP20 (7 Hz)) and fast-theta–alpha- (SP13 (main peaks at 5.5 and 10.5 Hz)) rhythmical segments when compared with lorazepam ($P < 0.0017$–$P < 0.00012$ for different channels). EEG/MEG during lorazepam was characterized by larger percentage of delta- (SP25 (2.5 Hz)), slow-theta- (SP18 (4 Hz)), delta–slow-theta- (SP22 (2.5 and 4 Hz)) and delta-beta- (SP26 (2.5 and 20.5 Hz)) rhythmical segments when compared with placebo ($P < 0.0026$–$P < 0.000001$ for different channels). The reaction to lorazepam was opposite to the group in subject S8 (Fig. 2).

Conventional ‘energetic’ estimation (mean spectral power) of the lorazepam-related EEG/MEG spectral changes revealed increase power in the slow (2–4.5 Hz) and fast (21–30 Hz) wavebands while reducing power in the mid-range (6.5–12 Hz) ($P < 0.0001$; see Fig. 2, insertions).

Although the lorazepam effect was similar for eyes closed and open conditions, the eyes opening modulated observed effect by increasing in EEG/MEG during placebo the number of segments with delta–slow-alpha (SP15 (2.5 and 8.5 Hz)), slow-theta–slow-alpha (SP11 (4 and 8.5 Hz)), and delta–slow-theta–slow-alpha (SP28 (2.5, 4.5 and 8.5 Hz)) activity ($P < 0.0026$–$P < 0.00012$ for different channels; not shown).

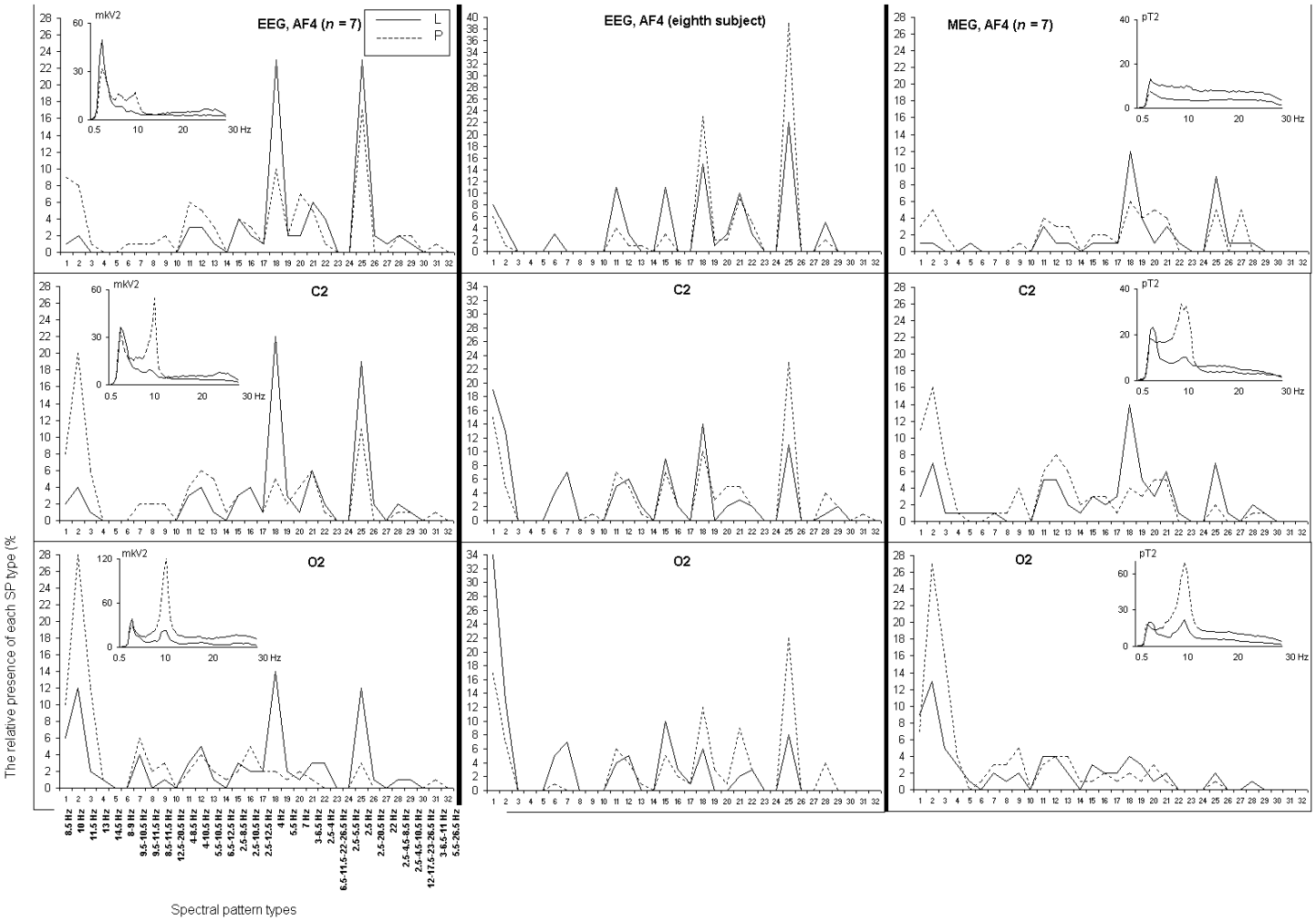

Figure 2. Classification profiles (CP) for EEG and MEG (averaged across 7 subjects, n = 35) and the subject S8 (n = 5) during lorazepam and placebo (closed eyes). O_2, C_2 and AF_4 locations are presented. The x-axis displays the labels (numbers) of the standard spectral patterns (SP) from 1 to 32 and their main frequency peaks. The y-axis displays the share of the corresponding SPs in the percentage from the total number of the classified SPs. A line graphic was chosen instead of a bar for the ease of comparison. (Note that x-axis consists of 32 discrete values, all the in-between values are meaningless). In the insertions conventional power spectra for EEG and MEG are presented.
L = lorazepam; P = placebo.

Even though the microstructural organization of EEG and MEG was similar, lorazepam effect for EEG was more pronounced than for MEG ($P < 0.001$) (Fig. 2).

Spatial distribution of spectral patterns was generally consistent with those from earlier studies. Thus, a significant ($P < 0.0017$) increase for alpha- and decrease for delta- and theta-rhythmical EEG/MEG segments in frontal-to-occipital direction was observed (Fig. 2). At the same time, the absence of inter-hemisphere asymmetry for lorazepam effect was demonstrated for both EEG and MEG estimates.

The main lorazepam effect described above was detected in the majority of EEG/MEG locations. Table 1 illustrates how many EEG channels were characterized by observed lorazepam effect for eyes-closed and eyes-open conditions. The lorazepam effect, in particular decrease of the percentage of alpha, fast-theta and fast-theta–alpha segments and increase of delta, slow-theta and delta-beta segments, was typical for more than 65% of EEG channels for eyes closed and open conditions (Table 1). If the Bonferroni correction is relaxed, then this value rises to 70-100% (for different SP types). Despite the different sensitivity of MEG and EEG to the underlying currents, MEG showed very similar results.

Some SP types revealed specificity in accordance with medication: they appeared in the EEG/MEG only either during lorazepam or during placebo. Lorazepam abolished SP8 (9.5 and 11.5 Hz), SP9 (8.5 and 11.5 Hz), SP13 (5.5 and 10.5 Hz) and SP14 (6.5 and 12.5 Hz). In the placebo condition, SP26 (2.5 and 20.5 Hz) was systematically absent, but appeared by lorazepam infusion in all subjects (not shown).

Both in EEG (Table 2a) and in MEG (Table 2b), there was more polyrhythmic activity (PA), larger non-stability of CPs (NSCP) and more homogenous CPs (NHCP) during lorazepam when compared with placebo ($P < 0.0026$–$P < 0.000001$ for eyes closed and open conditions). Surprisingly, the subject S8 shared this behavior of indexes, though its direct spectral estimations (see above) showed the opposite effect to the group (Table 2c).

3.2 The causes of changes in the power of EEG/MEG beta activity after lorazepam administration

In contrary to previous reports (Greenblatt et al., 1989; Breimer et al., 1990; Mandema et al., 1992; Van Steveninck et al., 1993) and conventional spectral estimations in the present study,

no increase of beta activity by lorazepam was observed in the short-term spectral analysis, except for the appearance of the SP26 (2.5 and 20.5 Hz) in EEG/MEG for lorazepam (Fig. 2). Further, this SP type characterized not more than 1.5% of the total number of segments in 1-min EEGs/MEGs, suggesting that this activity cannot be detected in the averaged power spectrum obtained by conventional spectral analysis.

Table 1. The number of EEG channels which were characterized by lorazepam effect for each spectral pattern type separately. Data averaged across 7 subjects (n = 35).

Closed eyes

Rhythm	SP	Frequency (Hz)	L < P s.s.		L < P n.s.	L > P s.s		L > P n.s.	no diff.	no SP P	no SP L
	1	8.5	43	58	15				1		
	2	10	52	59	7						
	3	11.5	43	59	16						23
α	4	13	10	19	9				1	39	53
	7	9.5-10.5	16	44	28				5	10	37
	8	9.5-11.5	41	51	10					8	57
	9	8.5-11.5	56	59	3						58
	12	4-10.5	14	54	40				5		
θ-α	13	5.5-10.5	43	54	11				5		
	14	6.5-12.5	21	34	13				1	24	58
Δ–α	16	2.5-10.5	21	54	33				5		
θ	20	7	38	57	19				2		11
θ-α	11	4-8.5	21	42	21	3	11	8	6		
Δ–θ–α	29	2.5-4.5-10.5	3	31	28		4	4	24		
Δ–α	15	2.5-8.5	6	24	18		20	20	15		
	17	2.5-12.5	1	18	17	3	8	5	33	3	2
Δ–θ–α	28	2.5-4.5-8.5		3	3	10	32	22	24		
θ	18	4				59	59				
	21	3-6.5				4	31	27	28		
Δ–θ	22	2.5-4				40	53	13	6		
Δ	25	2.5				38	58	20	1		
Δ–β	26	2.5-20.5				46	51	5	4	56	4

Open eyes

Rhythm	SP	Frequency (Hz)	L < P s.s.		L < P n.s.	L > P s.s		L > P n.s.	no diff.	no SP P	no SP L
	1	8.5	52	59	7						
	2	10	55	59	4						
	3	11.5	38	50	12					9	9
α	7	9.5-10.5	1	9	8				2	48	57
	8	9.5-11.5	20	24	4					35	59
	9	8.5-11.5	39	41	2					18	59
	11	4-8.5	20	41	21				17		
	12	4-10.5	38	58	20				1		
θ-α	13	5.5-10.5	42	57	15				2		45
	14	6.5-12.5	17	26	9				5	28	54
Δ–α	15	2.5-8.5	37	54	17				5		
	16	2.5-10.5	5	46	41				13		
	19	5.5	8	41	33				18		
θ	20	7	48	59	11						
	21	3-6.5	10	45	35				14		
Δ–θ–α	28	2.5-4.5-8.5	2	33	31				26		
Δ–θ–α	29	2.5-4.5-10.5	23	29	6		3	3	26	1	26
α	4	13	3	4	1	1	4	3	8	43	43
Δ–α	17	2.5-12.5	1	4	3	3	38	35	15	7	1
θ	18	4				42	55	13	4		
Δ–θ	22	2.5-4				35	43	8	16		
Δ	25	2.5				57	59	2			
Δ–β	26	2.5-20.5				40	54	14	3	49	2

Abbreviations:

L - lorazepam, P - placebo, s.s. - statistically significant, n.s. - nonstatistically significant, no diff. - no difference, no SP - given spectral pattern did not exist in particular number of channels. Numbers in the squares represent the total number of EEG channels which satisfy particular condition: L<P and/or L>P. "SP" column represents the label of spectral pattern type; "Hz" column represents frequency of the main peaks for each spectral pattern type. Gray color indicates only those spectral patterns for which the lorazepam effect was statistically significant in more than 65% of EEG channels.

Table 2. General characteristics of the classification profiles.
Data averaged across 59 EEG/MEG channels for all subjects (except subject S8)

a (EEG)

	Closed eyes		Open eyes	
	Lorozepam	Placebo	Lorazepam	Placebo
% of polyrhythmic spectral patterns	11.1+-7.2	5.9+-5.7	10.0+-6.6	6.4+-7.1
Non-homogeneity index	30.7+-5.3	38.4+-8.2	28.2+-6.5	38.9+-6.3
Non-stability index	28.2+-5.2	26.2+-4.1	28.8+-4.3	26.4+-5.4

b (MEG)

	Closed eyes		Open eyes	
	Lorozepam	Placebo	Lorazepam	Placebo
% of polyrhythmic spectral patterns	42.3+-12.2	24.8+-13.2	45.5+-9.4	31.1+-7.1
Non-homogeneity index	30.1+-3.8	32.3+-4.1	28.7+-4.1	34.1+-3.5
Non-stability index	34.0+-3.5	29.9+-3.4	34.5+-3.4	32.8+-2.2

c (subject S8)

	Closed eyes		Open eyes	
	Lorozepam	Placebo	Lorazepam	Placebo
% of polyrhythmic spectral patterns	4.1+-4.4	0.5+-1.2	6.5+-5.6	0.4+-0.7
Non-homogeneity index	33.9+-7.2	40.3+-5.2	33.5+-5.9	38.1+-5.0
Non-stability index	28.4+-4.6	25.2+-2.9	27.4+-4.1	24.6+-2.9

Conventional spectral analysis uses averaging procedures to obtain EEG power spectrum averaged out over extended periods of time and/or broad fixed frequency bands. The beta effect in the averaged power spectrum may originate from the averaged EEG segments with polyrhythmic activity. Table 2a and b illustrates that EEG/MEG segments with polyrhythmic activity (in terms of SPs) were presented in classification profiles more during lorazepam than during placebo. Also

spectral pattern 26 (2.5 and 20.5 Hz) may contribute to the beta effect in the average power spectrum. To check this hypothesis, all polyrhythmic SPs and SP26 were averaged separately and together for each EEG channel (Fig. 3).

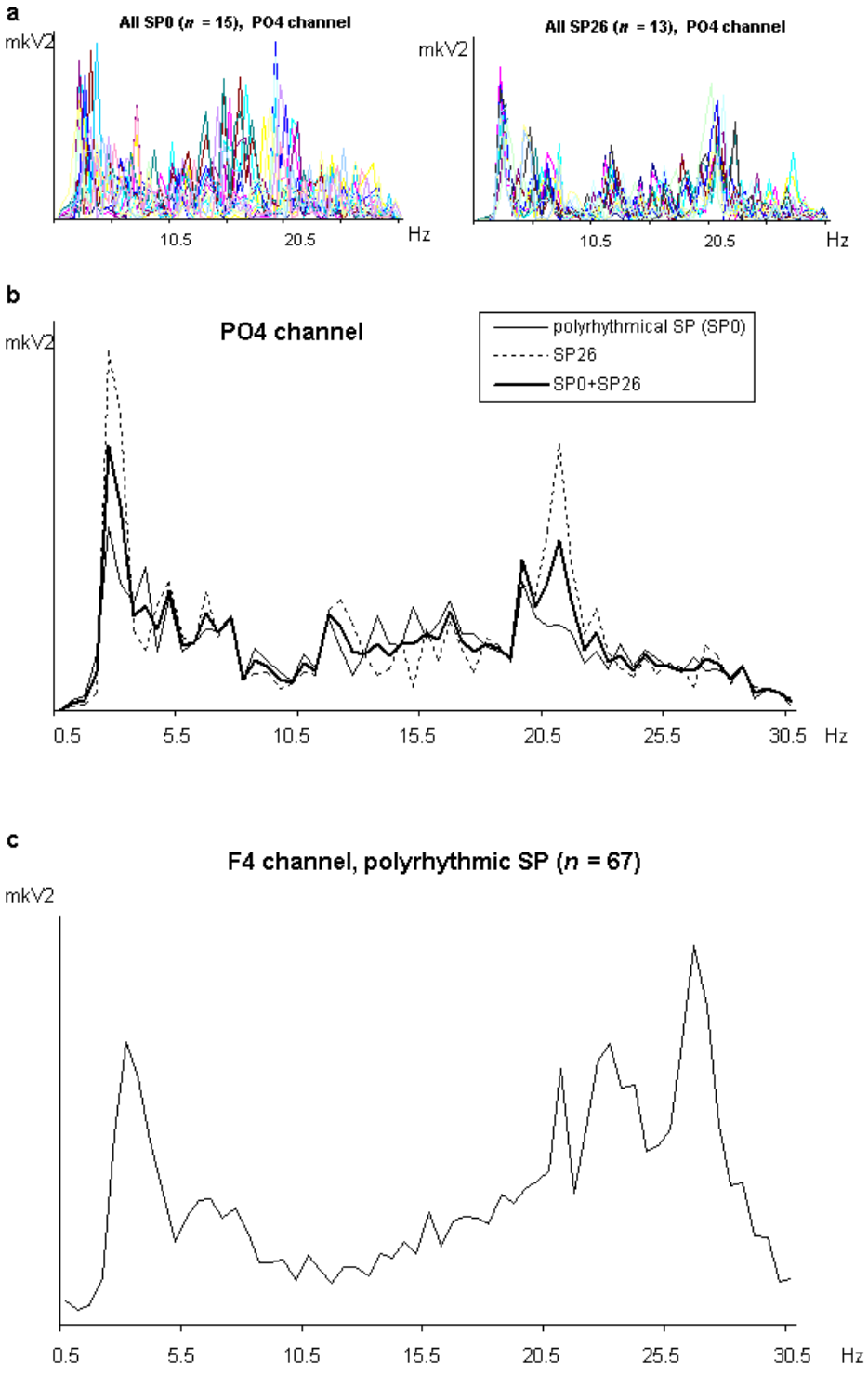

Figure 3. Example of individual and averaged EEG spectral patterns (SP). (a): individual SPs which were found in PO_4 channel are presented superimposed (left – polyrhythmic SPs (SP0); right – SP26 (main peaks at 2.5 and 20.5 Hz)). (b): averaged SPs for polyrhythmic SPs and SP26 which were averaged separately and together (PO_4 EEG channel). (c): averaged SP for polyrhythmic SPs (F_4 EEG channel).

Figure 3a illustrates as an example individual polyrhythmic SPs and SP26, which were found for PO_4 channel for one subject. Figure 3b represents averaged power spectra for polyrhythmic SPs (thin line) and SP26 (doted line) averaged separately and together (thick line) for PO_4 EEG channel. Figure 3c represents averaged power spectrum for polyrhythmic SPs for F_4 EEG channel (SP26 were not detected in this channel). Considerable concentration of averaged spectral power in delta and beta frequency bands was demonstrated, supporting our hypothesis.

3.3. Dynamics of temporal stabilization of the spectral patterns under the lorazepam influence

Since averaged power spectrum constitutes a 'static' picture which eliminates dynamic aspects of EEG/MEG transformations (Fig. 2, insertions), temporal characteristics of EEG/MEG under drug influence remain a mystery. Hence, the goal of current section was to study the dynamics of temporal characteristics of the spectral patterns under the lorazepam influence. Temporal stabilization of SP types was evaluated by computing the average number (for all EEG/MEG locations) of successive *m* EEG/MEG epochs of the same SP type (including polyrhythmic spectra – the type "0"), where *m* is the range from 1 to 149 and was described as a "block". In this case the particular block length reflects the particular period of temporal stabilization of brain oscillations. The results of this analysis for EEG and MEG are summarized in the Figure 4.

The effect of the temporary stabilization of SPs in EEG both for eyes closed and open conditions was almost identical for lorazepam and placebo having expected common characteristic: this index decreased as the length of block increased. At the same time, placebo condition was characterized by greater index values for small periods of temporal stabilization ($P < 0.0026$–$P < 0.000001$ for different block lengths) and smaller index values for large periods of temporal stabilization ($P < 0.0026$–$P < 0.000001$ for different block lengths) when compared with lorazepam (Fig. 4a). Similar results were obtained for MEG (Fig. 4b). Again, the subject S8, in

spite of the opposite to the group effect according to direct spectral estimations, showed similar index value (Fig. 4c).

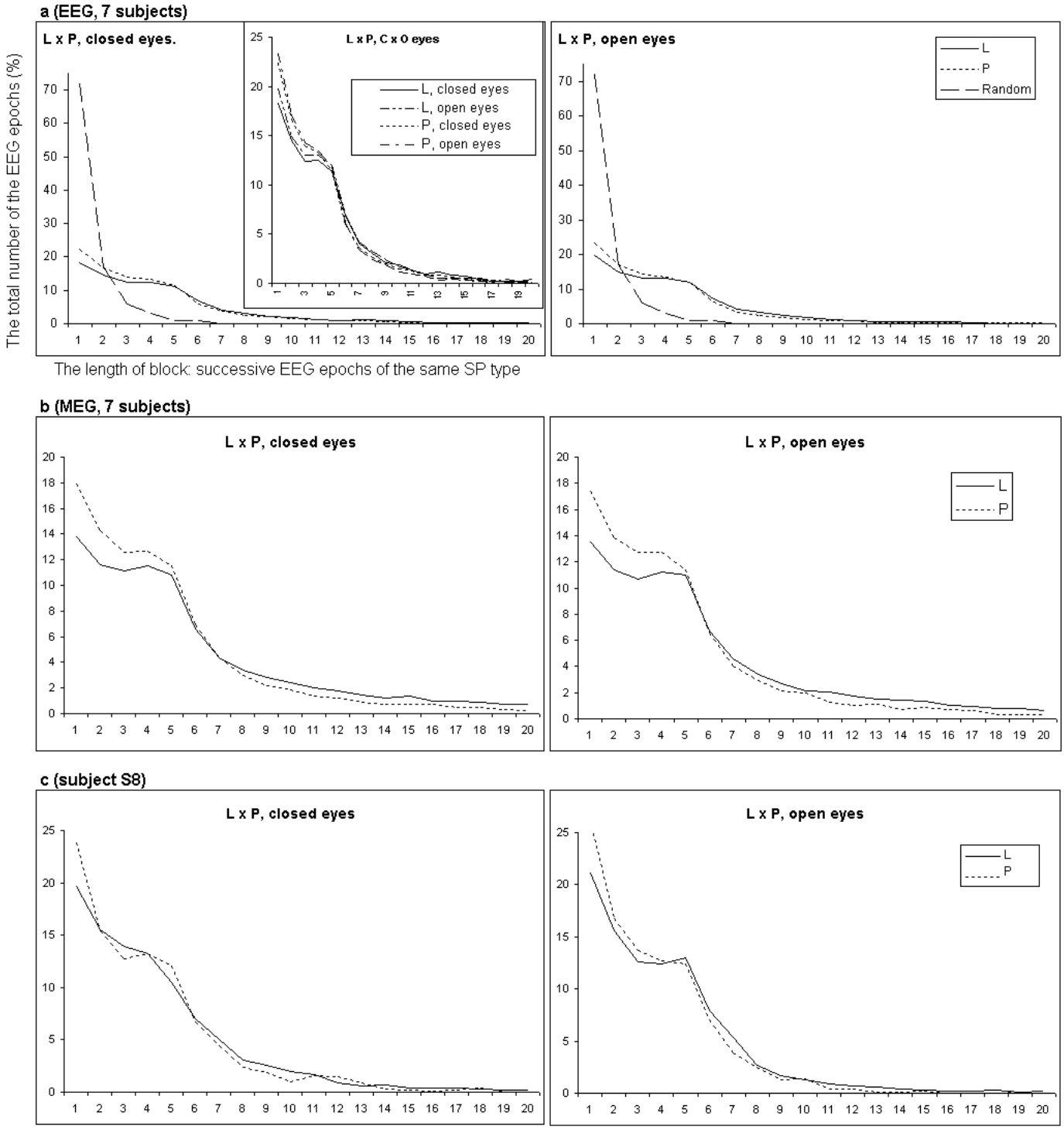

Figure 4. The average number (for all EEG/MEG locations, $n = 59$) of successive m EEG/MEG epochs of the same SP type (including polyrhythmic spectra) (the y-axis), where m is the range from 1 to 149 (the x-axis). The values are presented as a percentage of the total number of the epochs in all EEG/MEG recordings, for 7 subjects ($n = 5215$) and for subject S8 ($n = 745$).
"Random EEG" = EEG which natural sequence of spectral pattern types has been completely removed in each individual channel.
At the insertion, the EEG data presented superimposed in one graph in order to illustrate influence of eyes opening.

To illustrate the effect of the eyes open condition, the same data were presented superimposed on one graphic at the insertion (Fig. 4a). The opening of the eyes (for both lorazepam and placebo) resulted in an increase of the number of the individual EEG segments that were involved in the small periods of temporal stabilization and in a decrease of the number of the individual EEG segments, which were involved in the large periods of temporal stabilization when compared with eyes closed conditions ($P < 0.0026$–$P < 0.000001$ for different block lengths) (Fig. 4a).

However, it is obvious that even in the absence of any correlation between the EEG/MEG SPs there should be a temporary stochastic stabilization of the SPs, which may reflect merely occasional combinations of SP types. As control for the neural origin of temporal dynamics of SPs, surrogate data (an EEG with a random mix of different SP types separately for each channel) were used. From Figure 4a it can be seen that the actual EEG data substantially differed from the "random EEG". An excessive increase in the number of blocks of length 1 for "random EEG" may indicates a stochastic process.

Note that the analysis presented above could not reveal the dependence between the periods of temporal stabilization and the type of SPs. In other words, do specific type of brain oscillations (in terms of SPs) maintain a particular period of temporal stabilization? Therefore, we analyzed the maximum periods of temporal stabilization for all SP types, which were found in CPs for lorazepam and placebo (both for eyes closed and open conditions) (Fig. 5). The maximum periods of temporal stabilization for SP types presented in the Figure 5 as block length were recalculated in time-scale. This analysis showed that the brain "maintains" the stabilization period of neural activity for lorazepam between 2.8 and 7.5 sec (for different SPs, eyes closed and open conditions) (Fig. 5). For placebo, this range was somewhat narrower: 3.6–6.7 sec (for different SPs, closed and open eyes). Moreover, for placebo, all SPs with fast-theta, delta-alpha, fast-theta–

alpha and alpha activity were characterized by larger maximum periods of temporal stabilization than for lorazepam ($P < 0.0026$–$P < 0.000001$ for different SPs, eyes closed and open conditions) (Fig. 5). At the same time, for lorazepam, all SPs with delta, slow-theta, delta–slow-theta, delta-beta and with polyrhythmic activity were characterized by larger maximum periods of temporal stabilization than for placebo ($P < 0.0026$–$P < 0.000001$ for different SPs, eyes closed and open conditions) (Fig. 5). The duration of such periods for "random EEG" (an EEG with a random mix of different SP types) was substantially lower than in the actual EEG and reached up to 2.3–2.6 sec (for different SP types) (Fig. 5).

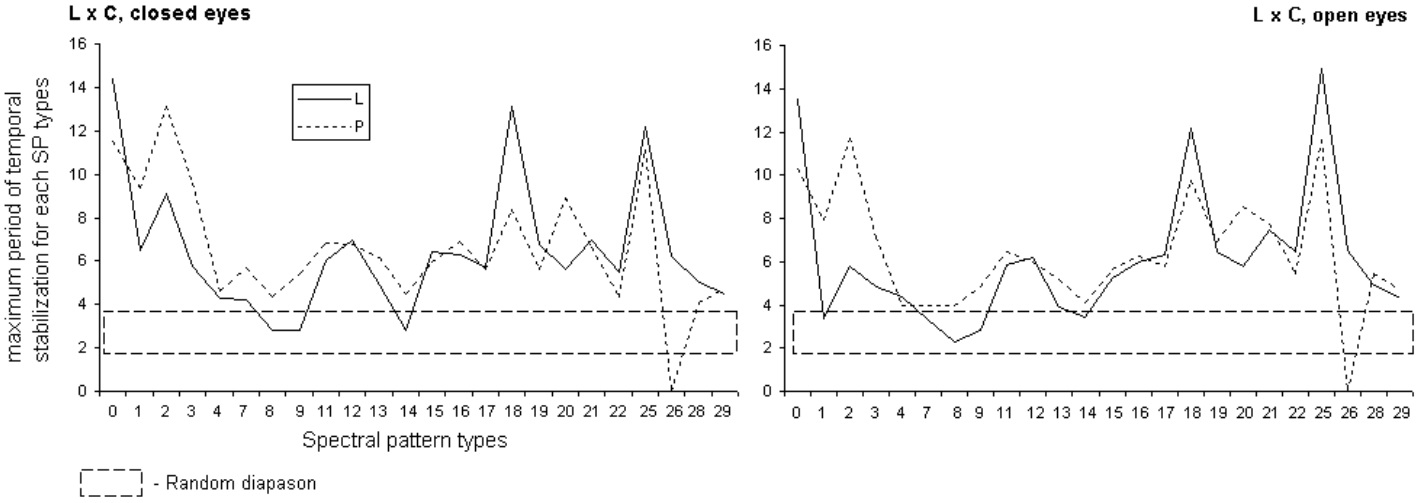

Figure 5. The maximum periods of temporal stabilization (averaged across 7 subjects) for all spectral pattern types, which were found in the EEG classification profiles during lorazepam and placebo (both for eyes closed and open conditions). The *x*-axis displays the labels (numbers) of the EEG spectral patterns (SP) corresponding to the standard SP set (including polyrhythmic spectra – type "0"). The *y*-axis displays the maximum periods of temporal stabilization for each SP types (in terms of block length – *m* EEG epochs follow in succession without SP type change, where *m* is the range from 1 to 149). Data averaged for all EEG locations ($n = 59$).
Horizontal dotted line bar represents random range of the maximum periods of temporal stabilization for "Random EEG" (EEG whose natural sequence of spectral pattern types has been completely removed in each individual channel).

3.4. Comparison with conventional spectral analysis and averaging procedures

The main effect of lorazepam that was detected using conventional spectral analysis was a decrease of alpha activity and an increase of delta and theta activity when compared with placebo (Fig. 2, insertions), at a first sight suggesting that this effect corresponds to the entire EEG being analyzed (~100% effect). However, this does not imply that this total power spectrum characterizes each of the individual power-spectra for each EEG segment. In fact, and as explored in our early work (Fingelkurts et al., 2003) and in the present one, this is not the case.

In order to estimate whether the averaged power spectrum characterized the whole of a given EEG/MEG, elemental calculations were performed. The total amount (in %) of EEG segments with "pure" delta, theta and alpha activity under lorazepam condition was calculated separately (for the occipital, central and frontal channels) using figure 2 (Fig. 6,A). The summation of the percents of SPs of "pure" delta, theta and alpha activity reflects only the (share) amount of 48-52% of EEG signal (for the different channels) and this relatively small share account for the averaged power spectrum of the whole signal. Hence, an as large share of EEG activity is "invisible" to the averaged power spectrum in conventional spectral analysis: [100% - (48-52%)]. Dominant spectral components determine the total picture of averaged EEG spectrum only due to their energetic predominance.

The comparison of averaged power spectra for lorazepam and placebo "reveals" the effect of lorazepam. However, and for the same reason that the averaged power spectrum does not characterize the whole of the signal, the arithmetic difference (its sign or its modulus) between the averaged power spectra of lorazepam and placebo cannot characterize the individual parts of the signal. Similarly to the earlier reasoning, the effect of lorazepam can be precisely calculated by obtaining the difference of percentages of the individual SPs of lorazepam and placebo, also for the "pure" delta, theta and alpha activity (Fig. 6,B).

The performed calculations showed that the amount of EEG affected by lorazepam which is visible for conventional spectral analysis ranges between 41% and 61% (for the different channels). This means that by using conventional spectral analysis we don't know what happens in 39-59% (*u*) of EEG (for the different channels) in this study. It may be assumed that there is a portion of the signal which is not affected by lorazepam at all. The obtained results support this supposition (Fig. 6,B). Thus, 5-20% (*v*) (for the different channels) of EEG individual segments

contribution to the EEG classification profile during the lorazepam influence or with placebo was the same – lorazepam did not affect some set of SPs. By subtracting this amount from the portion of EEG which is invisible for conventional spectral analysis, it is possible to obtain the exact amount of the EEG (19-51% (*u*-*v*) for the different channels) which is still affected by lorazepam, but not visible for conventional spectral analysis (Fig. 6,B). These EEG segments are characterized by several rhythmical components from different frequency bands (Fig. 2).

A

Rhythm	SP type i, SP_i	$\sum SP_{Li}$ (%) O2	C2	AF4
alpha	{1,2,3,4,5,6,7,8,9}	25	7	3
theta	{18,19,20,21}	15	23	20
delta	{25}	12	18	6
amount of EEG characterised by averaged power spectrum	$\sum_{alpha,theta,delta} SP_{Li}$	52	48	51
amount of EEG not characterised by averaged power spectrum	$1 - \sum_{alpha,theta,delta} SP_{Li}$	48	52	49

B

L-P	Rhythm	SP type i, SP_i	$\sum \lvert SP_{Li} - SP_{Pi} \rvert$ (%) O2	C2	AF4
- ↓	alpha	for every i of rhythm such L-P<0 (*)	37	33	15
+ ↑	theta	for every i of rhythm such L-P>0 (*)	13	20	20
+ ↑	delta	for every i of rhythm such L-P>0 (*)	9	8	6
0 =	*all-range*	for every i, such L-P=0	5	20	8
amount of EEG affected by lorazepam, visible through conventional spectral analysis		$\sum_{alpha,theta,delta} \lvert SP_{Li} - SP_{Pi} \rvert$	59	61	41
amount of EEG not visible through conventional spectral analysis		$(u) = 1 - \sum_{alpha,theta,delta} \lvert SP_{Li} - SP_{Pi} \rvert$	41	39	59
amount of EEG not affected by lorazepam and not visible through conventional spectral analysis		(v)	5	20	8
amount of EEG affected by lorazepam, and not visible through conventional spectral analysis		$u - v$	36	19	51

(*) - Inside the group of SP types of each rhytm, the direction of change was consistent for all SP types.

Figure 6. Comparison of results of short-term spectral pattern analysis with the results of conventional spectral analysis.
(A) Amount of EEG characterized by averaged power spectrum (in percent) for lorazepam, calculated from the values in figure 2 (see classification profile for lorazepam). (B) Amount of EEG affected by lorazepam (in percent), calculated from the values in figure 2 as summations of differences for each particular spectral pattern between lorazepam and placebo.
L = lorazepam; P = placebo; SP = spectral pattern; SP_i = spectral pattern of type *i*; SP_{Li} = spectral pattern of type *i* for lorazepam; SP_{Pi} = spectral pattern of type *i* for placebo; L-P = arithmetic difference of percentages of spectral patterns between lorazepam and placebo; O2, C2, AF4 = EEG channels; arrows indicate the direction of changes.

Neither the amount of EEG which is invisible for conventional spectral analysis (up to 59% of the total EEG), nor the portion of the signal which is not affected by lorazepam (up to 20% of the total EEG) and amount of the EEG which is characterized by several rhythmical components from different frequency bands and affected by lorazepam (up to 51%) can be detected by conventional spectral analysis in this study.

Hence, conventional spectral analysis based on averaging procedures can account the lorazepam effect but only for 41-61% (for the different channels) of the currently studied EEG. Also, the averaged power spectrum characterized only 48-52% (for different channels) of the total signal. Up to 50% of the signal remains “invisible” for such approach (Fig. 6). The adaptive classification analysis of individual short-term spectral patterns suggested in the present paper permits the study of 100% of EEG/MEG transformations in detail.

4. Discussion

4.1. Methodological aspects

Although pharmacological effects of benzodiazepines on brain dynamics have been widely studied, in most cases data has been averaged over extended periods of time and/or fixed frequency bands. Here we used adaptive classification analysis of short-term spectral patterns (see section 2.4. Data processing). This analysis revealed changes in the total amount of the time (percentage of EEG/MEG segments) that particular type of brain oscillations was on, rather than the changes in its amplitude or power.

Although EEG and MEG measures revealed very similar results, lorazepam effects in EEG were more pronounced than in MEG (Fig. 2). This difference is due to the different sensitivity of EEG and MEG. MEG is more sensitive to tangentially oriented cortical sources only, whereas EEG detects also radially oriented and deep sources (Hämäläinen et al., 1993).

4.2. Main EEG/MEG effect induced by lorazepam

We observed a significant decrease in the percentage of EEG/MEG segments with alpha activity and an increase in the percentage of EEG/MEG segments with delta and theta activity (Fig. 2), confirming the results of conventional spectral analysis and previous reports of lorazepam effects (Link et al., 1991; Entholzner, 1995). However, results of the present study substantially extended previously known data: lorazepam not only decreased power in alpha and increased power in delta and theta rhythms, but in fact, also decreased the number of EEG/MEG segments with fast-theta, delta-alpha, fast-theta–alpha and alpha activity, and increased the number of EEG/MEG segments with delta, delta–slow-theta, delta-beta, slow-theta and polyrhythmic activity when compared with placebo (Table 1). Moreover, using short-term spectral analysis it was demonstrated that lorazepam effect is typical for only 41-61% (for different channels) of a given EEG (Fig. 6), and averaged power spectrum characterized only 48-52% (for different channels) of a total signal.

In contrast to the previous data, the results of this study demonstrated the interactive nature of multiple brain oscillations and changes in microstructural organization of EEG/MEG during lorazepam administration. The interactive nature of multiple brain oscillations is in line with T.H. Bullock work (Bullock, 1997), where he reported the lack of independence between widely different frequency components from 0.5-30 Hz frequency range. Cellular mechanism for explanation of how can different types of oscillations coexist in the same network was suggested (Destexhe, 2000). By changing the resting level of thalamic neurons, the same thalamocortical circuits would be capable of generating low-frequency oscillations, as well as fast oscillations. The model also predicts that the kinetics of GABA inhibitory postsynaptic potentials as well as the intrinsic properties of reticular cells are critical in determining the frequency of oscillations (Destexhe et al., 1993). Thus, intrinsic neuronal mechanisms would dominate for generating the slow waves (0.5-4 Hz), whereas synaptic interactions with cortical and the thalamic reticular

nucleus would be required for faster oscillations in the frequency range 7-14 Hz. (Lytton et al., 1996). From this model it is clear why lorazepam affects oscillations in both frequency ranges. Additionally, $GABA_A$ receptor mediated inhibition has different roles in the network dependent on the target neuron. Inhibiting principal cells will thus reduce network excitability, whilst inhibiting interneurons will increase network excitability (Semyanov, 2003).

Since the main effects described in the present paper have been observed in the majority (> 65%) of EEG/MEG locations it may be suggested that distributed neuronal networks were affected by lorazepam. This supposition is supported by the work of Volkow with coworkers (Volkow et al., 1995) where it was shown that lorazepam significantly decreased whole brain and regional brain metabolism. Distributed EEG/MEG effects and metabolic response to lorazepam could reflect the result of the interaction with heterogeneously distributed benzodiazepine receptor subtypes in brain (Montpied et al., 1988; Semyanov, 2003). Strong metabolic changes with lorazepam in thalamus (Volkow et al., 1995) may also contribute to distributed EEG/MEG effects of lorazepam through widespread influences on the activity of the cerebral cortex (Carpenter and Sutin 1983). The influence of reference scheme which was used in this study may be ruled out, since MEG data which is strictly reference-free demonstrated the same result as EEG data. Moreover, the occipital and frontal regions clearly revealed differently pronounced lorazepam effect in EEG/MEG (Fig. 2).

The strength of lorazepam effect can be estimated by the percent-share of the whole signal which was not affected by the drug: the lower this percentage, the stronger the effect of lorazepam. Thus, the strongest effect of lorazepam was observed in posterior part of the head (Fig. 6). This finding is supported by the work of Volkow et al. (1995) where they demonstrated that the largest metabolic changes with lorazepam were in the occipital cortex. The occipital cortex is characterized by a high density of various benzodiazepine receptors subtypes (Inoue et al., 1992), which may explain their high sensitivity to the actions of benzodiazepine agonists.

4.3. Functional significance of different brain oscillations

Brain oscillations within theta band.

Lorazepam has a general sedative effect (Pohlman et al., 1994; Swart et al., 1999); and present EEG/MEG changes are in line with reported EEG changes during sedation (Entholzner et

al., 1995; Crippen, 1997). In accordance with general neurophysiological notions, observed EEG/MEG deceleration must be a reflection of certain inhibitory processes (Lazarev, 1998).

What is the mechanism of EEG/MEG slowing under the influence of lorazepam? Like other benzodiazepines, lorazepam facilitates $GABA_A$ neurotransmission in the brain (Korpi et al., 2002). $GABA_A$ interneurons contain two separate subclasses: $GABA_{A,fast}$ and $GABA_{A,slow}$ interneurons (Banks et al., 1998). $GABA_{A,slow}$ cells are distributed within the hippocampal formation that may contribute to the theta rhythm (White et al., 2000). Besides that, GABAergic inhibition plays a fundamental role in the timing of high-frequency oscillations (Whittington et al., 1996), and thus lorazepam prolongs synaptic inhibition. This can decrease the characteristic frequency of oscillations, imposing slow, high-amplitude waves in the EEG. Decreases in the EEG spectral frequency accompany decrements in cognitive function during the induction of anesthesia (Rampil, 1998). Moreover, $GABA_A$ receptors have an apparent role in synchronization and desynchronization of thalamocortical circuitry that contribute to the pathogenesis of epilepsy (Wong and Snead, 2001). Therefore, EEG/MEG slowing during lorazepam may also reflect anesthetic (Kennedy and Longnecker, 1996) and anticonvulsant (Alldredge et al., 2001) effects of this drug.

Destexhe and co-workers proposed a cellular mechanism in which 2-4 Hz oscillations (dominant inhibitory effect) invade the entire network through a mutual interaction between cortex and thalamus (thalamocortical loops) (Destexhe, et al., 1998; Destexhe, 2000). The model suggests that corticothalamic feedback must operate on the thalamus mainly through excitation of GABAergic thalamic reticular neurons, therefore recruiting relay cells essentially through inhibition and rebound (also see Blumenfeld and McCormik, 2000). It seems that lorazepam affects thalamocortical loops by activating GABAergic thalamic reticular neurons.

EEG/MEG slowing during lorazepam cannot be attributed to drowsiness at present study. Drowsiness is characterized by the presence of sleep spindles in EEG (Rechtschaffen and Kales, 1968). Neither visual analysis, nor spectral analysis (Table 1) of subjects' EEG and MEG during lorazepam revealed the increase of sleep spindles. Moreover, in contrary to sleep-inducing drugs such as zolpidem and midazolam (Durka and Blinowska, 2001; Durka et al., 2002), lorazepam did not increase the number of EEG/MEG segments with 12-15 Hz and did not decrease the number of EEG/MEG segments with 1-2 Hz in the present study (Table 1).

At the same time, the larger percentage of EEG/MEG segments with fast-theta for placebo when compared with lorazepam (in the present study) may relate to mechanisms of arousal (Mizuki, 1987).

Brain oscillations within alpha band.

Alpha waves from 8 to 13 Hz are the primary waveforms seen in awake subjects. In addition to confirming that alpha amplitudes are very sensitive to benzodiazepines (Koopmans et al., 1988; Van Steveninck et al., 1993), the present results extend this information: benzodiazepines not only decrease the amplitude of alpha rhythm but also reduce the total time when the brain generates this activity. Alpha waves respond readily to the changes in the brain's functional state, decreasing with tranquility (Crippen, 1997). Therefore, the decrease in the number of EEG/MEG segments with "pure" alpha activity and EEG/MEG segments with alpha components during lorazepam may reflect tranquility effect of this drug.

A decrease in global cerebral glucose metabolism by lorazepam (detected by positron emission-tomography) was reported by Volkow et al. (1993, 1995). Also, a decrease of EEG alpha power by lorazepam was significantly correlated with decreased glucose metabolism in the thalamus (Lange-Asschenfeldt et al., 2001). These findings suggest that alpha effects observed in the present paper may be caused by reduced brain metabolism.

Brain oscillations within beta band.

Increases in the EEG-beta amplitudes usually are used to quantify the effects of benzodiazepines (Greenblatt et al., 1989; Breimer et al., 1990; Mandema et al., 1992; Van Steveninck et al., 1993). However, in contrary to these reports, no increase of beta activity by lorazepam was observed in the present study. It was suggested and demonstrated here that changes in the beta frequency band (increase by lorazepam) that are present in averaged spectrum, most likely originated from averaged polyrhythmic activity, which is observed during lorazepam in a significantly more percentage than during placebo. This suggests that lorazepam does not increase activity of independent beta rhythm.

Mathematical modeling of natural network dynamics for inhibition-based rhythms also suggests that increased beta activity elicited by benzodiazepines cannot be observed in a natural network (Whittington et al., 2000). Thus, in the absence of drugs (normal condition), gamma

activity is manifest as a train of outward synaptic currents (IPSCs) mediated by $GABA_A$ receptor activation. Benzodiazepines increase the amplitude of each IPSC with comparably little effect on decay kinetics. The resulting frequency of the IPSC train is approximately halved. In the case of potentiated IPSPs, beta oscillations are only seen in isolated interneuron networks. With strong recruitment of excitatory neurons (natural network), the frequency of oscillation remains within the gamma band (Faulkner et al., 1998).

4.4. General characteristics and dynamical behavior of brain oscillations under the lorazepam influence

Dynamical indices were more sensitive for lorazepam than direct spectral estimations (Table 2, Fig. 4). Thus, subject S8, who had opposite to the group effect according to direct spectral estimations, had similar dynamical indices with the group. It was shown here that lorazepam caused decreased diversity (in terms of spectral patterns) of EEG/MEG signal and increase general instability of CP. Accounting for anesthetic effect of benzodiazepines (Kennedy and Longnecker, 1996), these results are consistent with the previous finding (McEwen and Anderson, 1975) that EEG activity during anesthesia is significantly less stationary than baseline activity.

A single EEG/MEG spectrum illustrates the particular integral dynamics of tens and hundreds of thousands of neurons in a given cortical region at a particular time period (Dumermuth and Molinari, 1987). Therefore, the absence of variance of a single spectrum during several consecutive analyzed epochs indicates that in a given cortical region the same macro-regimen of neuronal pool activity is maintained during that period. This phenomenon of a temporal stabilization may be explained by stabilizing oscillatory patterns in the brain. Thus, EEG during lorazepam (eyes closed and open conditions) was characterized by longer periods of SP temporal stabilization than during placebo.

At the same time, in the present study the eyes-open condition “preferred” shorter periods of SP temporal stabilization than the eyes-closed condition (Fig. 4). Increased stabilization periods of SPs by lorazepam and eyes-closed condition suggest a reduction of brain information processing (Fingelkurts et al., 2003). Additionally we found that concrete parameters in the lifetime of each of the SP type are specifically related to the influence of lorazepam (Fig. 5).

Note that all these estimations differed significantly in the “random EEG” (EEG whose natural sequence of SP type has been completely removed in each individual channel), which reflect that the temporary stabilization of the main dynamic parameters of neuronal activity being non-occasional character (Fig. 4, 5).

It is therefore likely that the temporal variability/stability of EEG/MEG SPs provides additional information when characterizing lorazepam effects on CNS in terms of EEG/MEG correlates. Thus, suggested approach for EEG/MEG analysis that encompass interactive and dynamic nature of multiple brain oscillations can give a broader picture of drug effects in comparison to conventional methods and may be used as a complementary approach to classical spectral analysis. The usage of suggested approach may help to develop a more rational neuropsychopharmacology.

5. Conclusions

Results of the present paper not only supported previously obtained conclusions, but also revealed new aspects of lorazepam effects:

(a) Complex interplay of brain oscillations during lorazepam administration was observed. This interplay of brain oscillations presumably reflects the complex multidimensional neurodynamic structure of brain activity under lorazepam influence which is formed by certain balance of independent neurophysiological processes. (b) The lorazepam effect measured by EEG and MEG was very similar and was observed in more than 65% of EEG/MEG locations. (c) At the same time, known lorazepam effect was typical for only 40-60% (for different channels) of a given EEG. (d) Lorazepam administration significantly reorganized the microstructure of EEG/MEG signal. This suggests that temporal EEG/MEG characteristics may provide additional information on drug effects. (e) Lorazepam increased stabilization periods of the spectral patterns reflecting a reduction of brain information processing. (f) Lorazepam caused no increase power in the independent beta rhythm.

Suggested approach, which permits a detailed description of brain oscillations including temporal and dynamic changes of brain activity, improves on the sensitivity of the conventionally used spectral estimates and opens new possibilities for researchers. Sensitivity of the approach becomes obvious in the three following cases:

(1) Apart from the higher sensitivity, a detailed description of SPs dynamics allows for a closer investigation of the causes of changes in the averaged power estimates. For example, we observe from the numerical values given in Figure 3, that the change of total power in beta range (averaged spectrum) after lorazepam administration results from a change in the number of polyrhythmic SPs occurrences per minute rather than change of the average beta amplitude.

(2) The EEG/MEG recordings of the subject S8 had opposite to the group effect according to direct spectral estimations under the influence of lorazepam (Fig. 2). Selective dynamical indices revealed the expected lorazepam effect, coherent with all the other subjects (Fig. 4, Table 2).

(3) In contrast to the conventional spectral analysis it is possible to estimate exact portion of EEG/MEG signal which is affected by the drug.

All these phenomena would be very difficult to detect using the conventional approach.

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