# Increased Local and Decreased Remote Functional Connectivity at EEG Alpha and Beta Frequency Bands in Opioid-Dependent Patients

Andrew A. Fingelkurts[1,2,3*], Alexander A. Fingelkurts[1,2,3], Reetta Kivisaari[4], Taina Autti[4],

Sergei Borisov[5], Varpu Puuskari[6], Olga Jokela[6], Seppo Kähkönen[2,3]

[1]*BM-SCIENCE – Brain and Mind Technologies Research Centre, P.O. Box 77, FI-02601, Espoo, Finland*
[2]*BioMag Laboratory, Engineering Centre, Helsinki University Central Hospital, P.O. Box 442 FIN-00290 Helsinki, Finland*
[3]*Cognitive Brain Research Unit, Department of Psychology, University of Helsinki, Finland*
[4]*Helsinki Medical Imaging Center, Helsinki University Central Hospital, Helsinki, Finland*
[5]*Center for Functional and Molecular Imaging, Georgetown University Medical Center, Georgetown University, Washington, USA*
[6]*Department of Psychiatry, Helsinki University Central Hospital, Helsinki, Finland*

## Abstract

*Rationale:* Although researchers now have a working knowledge of key brain structures involved in realization of actions of substance abuse and addiction, deeper understanding will require examination of network interactions between cortical neuronal assemblies and their subcortical tails in the effects of opioid dependence. *Objectives:* Given that repeated exposure to opiates initiates a widespread reorganization of cortical regions, we predict that opioid dependence would result in a considerable reorganization of local and remote functional connectivity in the neocortex. *Methods:* We applied the novel operational architectonics approach that enables us to estimate both local and remote functional cortex connectivity by means of EEG structural synchrony measure. *Results:* In twenty two opioid-dependent patients, we found the evidence that brain functional connectivity was indeed disrupted by chronic opioid abuse (i.e., the local functional connectivity increased and remote functional connectivity decreased in opioid abusers). This significant difference between "opioid" and "control" populations was the same for alpha and beta frequency bands. Additionally, significant negative relations between duration (years) of daily opioid abuse and the number/strength of functional connections in the posterior section of the cortex were found.

**Keywords:** addiction, brain interactions, cortex, metastable states, opiate, opioid dependence, structural synchrony, synchronization

**Introduction**

Advances in the understanding of the brain as a highly integrated and dynamic organ (Freeman et al. 2001; Fingelkurts and Fingelkurts 2004) provide a new perspective on substance abuse effects and addiction. Recent imaging studies suggest that reward-related behavior and drug addiction emerge from the dynamic activity of large neural networks rather than from any single brain structure. The functions of frontal cortex, temporal insula, thalamus (Goldstein and Volkow 2002), as well as nucleus accumbens (Noel and Gratton 1995), amygdala (Baxter et al. 2000), and sensorimotor structures (White 1989) in natural reward and addiction can be understood better in terms of the extended and functionally connected neural system within which they reside. Thus, although researchers now have a working knowledge of these key brain structures involved in the realization of actions of abused drugs (Robinson and Berridge 2000; Hyman and Malenka 2001), deeper understanding will require examination of network interactions between cortical neuronal assemblies (including their subcortical tails) in the effects of substance abuse and addiction. Indeed, previous studies show that drug-abuse craving may be associated with abnormal cortical processing (Ingmar et al. 2004).

Here, the electroencephalogram (EEG) provides a satisfactory measure for accessing temporal evolution of both local and remote neuronal interactions in the cortex at the millisecond scale (Nunez 2000; Freeman 2003). The network interactions revealed using EEG can be used to study the effects of abused drugs directly in the human brain and to investigate the long-term consequences of addiction on the brain. Although it is an important issue, there have been no explicit studies of local and remote functional connectivity in the brain of chronic opioid abusers. The research has been focused mostly on methadone maintenance patients and has been limited largely to indirect investigations (PET, MRI, fMRI, and magnetic resonance spectroscopy – MRS), where the set of brain areas associated with abused drug effects and addiction was established (for the review, see Volkow et al. 2004). However, to reveal the set of brain areas is not sufficient to prove whether the activated areas are actually functionally connected.

The present study was designed as a first part of the longitudinal research program aiming to explore the role of local and remote functional cortex connectivity in current opioid addicts entering the hospital unit for withdrawal and evaluation for methadone treatment program (other parts of this program will be published separately). To achieve the aim of this study, we applied the novel operational architectonics approach (Kaplan 1998; for the review and development, see Fingelkurts and Fingelkurts 2005) that enables us to estimate both local and remote functional cortex connectivity through EEG measure (Fingelkurts et al. 2004). According to this approach, the formation of a neuronal assembly is accompanied at the EEG level by a period of rhythmical

increase in the total potential, whereas a disruption of the neuronal assembly is characterized by a period of decrease in the total potential (John 2001; Koenig et al. 2002; Klimesch et al. 2005). As shown by Freeman, this common carrier wave generated by the neuronal assembly's interaction indeed conveys the EEG amplitude modulation patterns (Freeman 2000). Therefore, the periods of quasi-stationary EEG segments correspond to the temporary stable *local* microstates in brain activity, where neurons participating in the neuronal assembly are functionally synchronized (Nunez 2000). A neuronal assembly may be defined as a group of neurons that synchronize their activity to perform a specific computation (operation) required for a specific task (Varela 1995; Nunez 2000; Breakspear and Terry 2002). According to this cell assembly theory, the coordinated information could be extracted from the relations between multiple neurons in an assembly, whereby these relations are reflected in the neural synchrony (Singer and Gray 1995). Indeed, it has been shown that changes in such synchrony (a) are stimulus-frequency-specific, (b) follow the time course of ongoing stimuli, (c) are systematically mapped across the cortical surface (deCharms and Merzenich 1996), and (d) determine local (immediately under the EEG recording electrode) brain activity (Cook et al. 1998). Generally, this view is consistent with the currently suggested concept of 'functional source,' which is defined as the part (or parts) of the brain that contribute to the activity recorded at a single sensor, does not have to coincide with a well-defined anatomical part of the brain, and is neutral with respect to the problems of source localization and volume conduction (Stam 2005). For the detailed review of the neuronal assembly theory, see Fingelkurts and Fingelkurts (2005).

The *remote* functional connectivity among distinct neuronal assemblies is reflected at the EEG level in the synchronization of quasi-stationary segments (estimated by EEG structural synchrony, SS) between different EEG electrode sites (brain regions) in specific frequency bands (Kaplan et al. 1997, 2005; Fingelkurts et al. 2005a). As a result of such synchrony, the periods of short-term metastable brain states originate (for the reviews, see Kaplan 1998; Fingelkurts and Fingelkurts 2001, 2004). In the metastable regime of brain functioning, the individual parts of the brain exhibit tendencies of functioning autonomously at the same time as they exhibit tendencies of coordinated activity (Kelso 1995). In this context, opioid dependence may be conceptualized as a new metastable state around altered homeostatic levels in the brain. From this point of view, the 'addicted' brain should have characteristic differences in the functional connectivity from the intact brain.

Given that repeated exposure to opiates initiates a widespread reorganization of cortical regions (Hyman and Malenka 2001; Robinson et al. 2002) that mediate cognitive functions, including those associated with drug-seeking and drug-taking behavior that characterize

addiction (Robinson and Berridge 2000), we predict that systematic opioid exposure in abuse patients would result in a significant reorganization of local and remote functional connectivity in the neocortex.

**Materials and Methods**

*Subjects*

The study included a total of twenty two right-handed opioid-dependent patients, fourteen men and eight women aged between 21 and 46 years of age (mean, 33 ± 5 years) and 14 controls, six men and eight women aged 33 ± 5 years. Patients were hospitalized for 2 weeks in a drug-withdrawal unit before starting methadone maintenance therapy. Criteria for such therapy at Helsinki University Central Hospital included minimum age of 20 years, 4 years of documented i.v. opioid abuse, and failure of institutional or long-lasting out-patient withdrawal therapy, which also served as criteria for the present study inclusion. Exclusion criteria for methadone maintenance therapy were uncontrolled polysubstance abuse, physical or psychiatric illness, and alcohol dependence. In the present study, additional exclusion criteria for both patients and controls were major head trauma, chronic neurologic illness, or ongoing medication for neurologic symptoms.

All patients had abused opioids for 4–26 years (mean, 11 ± 5 years). Self-reported daily dose was 0.05–2 g for i.v. administration of heroin and 2-32 mg for i.v. administration of buprenorphine. Almost all patients reported irregular (episodic) use of cannabis, amphetamine, and alcohol for short periods earlier in their lives. Some patients reported use of benzodiazepines (15 patients), cannabis (5 patients), and amphetamine (5 patients) when heroin was not available. However, street buprenorphine and heroin were the only drugs used by the patients regularly (daily) for several years (at least 4).

Psychiatric diagnoses of patients and controls were explored using Structured Clinical Interviews I and II (SCID I and II) (First et al. 1994a, b), which afford detailed information according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (American Psychiatric Association 1994). All 22 patients met DSM-IV criteria for opioid dependence, whereas 15 patients met also DSM-IV criteria for benzodiazepine dependence. Patients fulfilled no other DSM-IV criteria aside from substance abuse on axis I; all met DSM-IV criteria of axis II diagnosis (American Psychiatric Association 1994) for personality disorders. The most common was antisocial, obsessive-compulsive, paranoid, borderline, narcissistic, schizoid, passive-aggressive, dependent, and depressive personality disorders. Controls did not fulfill any criteria for DSM-IV disorders on SCID I or II. Neuropsychologic tests (Wechsler Adult

Intelligence Scale; Wechsler 1993) showed normal intelligence in all subjects. Patients had no neurologic complaints. Controls were volunteers from the staff of the Institution, and no control had any experience with illegal drugs, but all had drunk alcohol on social occasions. However, none met criteria of abuse of or dependence on alcohol. The study was accepted by the Ethics Committee of the Helsinki University Central Hospital and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. All the subjects studied gave informed written consent before enrolling in the study.

*Trial design*

Before the study, patients gave the urine samples twice a week for a minimum of six weeks to exclude other substance abuse than opioids. The patients were investigated on the day of admission and all had abused opioids within twelve hours before EEG registration; the dosages were the patients' usual dosages. None of the patients had a withdrawal syndrome at the time of EEG registration, as verified by a Gossop test (Gossop 1990). Following electrode placement and instrument calibration, a subject (patient or healthy control) was seated in a comfortable chair in a dimmed registration room, and the experimental procedure was explained. The EEG recording was started at Noon. To reduce muscle artifacts in the EEG signal, a subject was instructed to assume a relaxed position and to avoid movements. A subject was instructed also to look straight in front of him/her (even though the eyes were closed). The behavior of a subject was observed on a TV monitor throughout the experiment. Each subject underwent five minutes EEG registration with eyes closed.

*EEG registration*

All recordings were performed in a magnetically and electrically shielded room (Euroshield, Eura, Finland) in the BioMag Laboratory, Helsinki University Central Hospital. EEG was recorded with a 60-channel EEG data acquisition system (Neuromag Vectorview, Helsinki, Finland) with a frequency band of 0.06 to 86 Hz (sampling rate 600 Hz). The nose electrode was used as reference. The impedance of each electrode was monitored for each subject with an impedance meter prior to data collection; this was always below 5 k$\Omega$. Vertical and horizontal electro-oculograms were recorded. The presence of an adequate signal was determined by visually checking each raw signal on the computer screen.

*Data processing*

EEG components containing artifacts due to eye blinks, significant muscle activity, and movements were automatically corrected by means of ICA (Independent Component Analysis) procedure (Hyvärinen et al., 2001). After removing artifact-related components, the back projection of remaining components originating from the brain was performed (Joyce et al., 2004). The procedure is implemented as "The FastICA package for MATLAB" and is freely available online http://www.cis.hut.fi/projects/ica/fastica/ .

A full EEG streams free from artifacts contained 5-min continuous signal (eyes closed) for each patient and control subject. EEG data were split into 2 distinct groups: "opioid" and "control." Further data processing was performed separately for each 1-min portion of the signal. Due to the technical requirements of the tools that were later used to process the data, EEGs from 20 electrodes ($F_{7/8}$, $F_z$, $F_{3/4}$, $T_{3/4}$, $C_{5/6}$, $C_z$, $C_{3/4}$, $T_{5/6}$, $P_z$, $P_{3/4}$, $O_z$, and $O_{1/2}$) were analyzed with a converted sampling rate of 128 Hz. After resampling and prior to the nonparametric adaptive segmentation procedure, each EEG signal was bandpass-filtered (Butterworth filter of the sixth order) in the alpha (8-13 Hz) and beta (15-21 Hz) frequency bands. Phase shifts were eliminated by forward and backward filtering. These frequency bands were chosen because it has been well documented that the most consistent changes in EEG of opioid addicts were observed in alpha and beta frequency ranges (for the review, see Polunina and Davydov 2004).

*Estimation of the local functional interrelations*

Local functional interrelations were estimated in two phases. At the *first phase*, the adaptive level segmentation of local EEGs was performed. Each 1-min EEG was segmented using method of identification of rapid transition processes (RTPs) in the EEG amplitude (*RTPseg*) tool (see Fingelkurts et al. 2006a). RTPs are the markers of boundaries between quasi-stationary segments in EEG. This method is based on the automatic selection of level-conditions in accordance with a given level of the probability of "false alerts" and carrying out simultaneous screening of all EEG channels (for details, see Kaplan et al. 2005; Fingelkurts et al. 2004). With this technique, the sequence of RTPs with statistically proven ($P<0.05$, Student *t*-test) time coordinates has been determined for each EEG channel individually for each 1-min epoch. The theoretical concepts behind this analysis are described elsewhere (Kaplan 1998; Kaplan and Shishkin 2000; Fingelkurts et al. 2005a).

At the *second phase*, after quasi-stationary segments (indexed by RTPs) were obtained, several characteristics (attributes) of segments (Kaplan and Borisov 2003) were calculated. These attributes reflect different aspects of local processes in the cortex and thus permit assessing the mesolevel description of cortex interactions (interactions within transient neuronal assemblies) through large-scale EEG estimates (Fingelkurts et al. 2004). The attributes are: (1) *Average amplitude* (A) within each segment (μV) – as generally agreed, indicates mainly the volume or size of neuronal population: indeed, the more neurons recruited into assembly through local synchronization of their activity, the higher will be the amplitude of corresponding to this assembly oscillations in the EEG (Nunez 2000; Klimesch et al. 2005). (2) *Average length* (L) of segments (msec) – illustrates the functional life span of neuronal population or the duration of operations produced by this population: since the transient neuronal assembly functions during a particular time interval, this period is reflected in EEG as a stabilized interval of quasi-stationary activity (Fingelkurts et al. 2004). (3) *Coefficient of amplitude variability* (V) within segments (%) – shows the stability of local neuronal synchronization within neuronal population or assembly (Truccolo et al. 2002). (4) *Average amplitude relation* (AR) among adjacent segments (%) – indicates the neuronal assembly behavior – growth (recruiting of new neurons) or distraction (functional elimination of neurons) (Kaplan and Borisov 2003). (5) *Average steepness* (S) among adjacent segments (estimated in the close area of RTP) (%) – shows the speed of neuronal population growth or distraction (Kaplan and Borisov 2003).

*Estimation of the remote functional connectivity*

Remote functional connectivity was estimated by calculation of the index of EEG SS. The index of structural synchrony (ISS) was estimated through synchronization of rapid transition processes (RTP) between different EEG channels (*RTPsyn*) tool (see Fingelkurts et al. 2006a). This measure reveals functional (operational) interrelationships between cortical sites different from those measured by correlation, coherence, and phase analysis (Kaplan et al. 2005; Fingelkurts et al. 2005a). In brief, each RTP in the reference EEG channel (the channel with the minimal number of RTPs from any pair of EEG channels) was surrounded by a short "window" (ms). Any RTP from another (test) channel was considered to coincide if it fell within this window. The ISS tends toward zero where there is no synchronization between the EEG segments and has positive or negative values where such synchronization exists (Fingelkurts et al. 2006a). Positive values indicate "active" coupling of EEG segments (synchronization of EEG segments are observed significantly more often than expected by

chance; *P*<0.05, random shuffling, computer simulation), whereas negative values mark "active" decoupling of segments (synchronization of EEG segments are observed significantly less than expected by chance; *P*<0.05, random shuffling, computer simulation). From a qualitative perspective, the coupling of EEG segments corresponds to the phenomenon of synchronization of brain operations or operational synchrony – OS (Fingelkurts and Fingelkurts 2001, 2005).

Using pair-wise analysis, SS was identified in several channels (more than two). These are described as operational modules – OM (Fingelkurts and Fingelkurts 2003, 2005). OM means that the set of the cortical areas participated in the same functional act during the analyzed period. The criterion for defining an OM was a set of EEG channels in which each channel forms a paired combination (with high values of ISS) with all other EEG channels in the same set; meaning that all pairs of channels in an OM have to have significant ISS. The number of cortical areas recruited in OM is described as "the order of areas recruitment."

*Statistics*

For each condition ("opioid" *vs.* "control" groups), group-EEG-segment-attributes averages and respective standard deviations were calculated in the following manner: a) At first and for each segment attribute, per-subject individual averages were calculated from the 5 epochs of 1-min EEG registrations (separately for each channel); b) For all subjects of the group-condition, the previously calculated per-subject average parameters were again averaged together, now aiming to characterize the group. The initial per-subject averaging prevents the error induction in the group statistics that would happen if the statistics would be calculated to the whole group subject pool directly. The per-subject averages permit to check if the results between the subjects are consistent for each group-condition, and only then if the consistency exists, it would be correct to average the group. All subjects in our study have very similar changes in the EEG segment attributes, what was reflected in very small values of standard deviations; these justify the pulling of all data of the group-condition together in order to characterize the group. As in the previous work (Kaplan et al. 2002; Kaplan and Borisov 2003; Fingelkurts et al. 2004), the comparison of the same segment attributes between different group-conditions was performed using Wilcoxon *t*-test.

The differences in the number and strength of SS EEG patterns between patients and controls were assessed using the Wilcoxon *t*-test as in the majority of the functional connectivity studies (for overview, see Weiss and Rappelsberger 2000). All SS-pair EEG patterns were divided into nine categories (short$_{left/right}$, short$_{anterior/posterior}$, long$_{left/right}$,

long$_{anterior/posterior}$, long$_{interhemispheric}$) separately for alpha and beta frequency bands. Pairs of EEG electrodes that have one or more electrodes between the "members" of the pair were classified as long-range connections (according to Weiss and Rappelsberger 2000). Since the absolute number of possible SS EEG pairs within each category was different, the percentage of the number of SS EEG pairs was calculated.

Separate computer maps of the ISS values were created for each subject and for each 1-minute EEG. The problem of multiple comparisons between maps cannot easily be overcome due to the large number of electrode pairs (Rappelsberger and Petsche 1988) in the SS maps. This problem is common to all studies which require multiple comparisons between maps (Weiss and Rappelsberger 2000). The comparisons that have been made should therefore be considered descriptive rather than confirmatory (von Stein et al. 1999). To have valid results and to overcome the multiple comparisons problem (for justification, see Appendix E in Fingelkurts et al., 2006a), and as we have done in our previous work, all pair combinations of EEG channels exhibiting statistically proven SS ($P<0.05$) were ranged in accordance with their rate of occurrence within all analyzed 1-min EEG epochs in each subject and across all subjects. Only the most frequently found combinations (not less than 85% occurrence in all epochs and all subjects) for the same experimental group ("opioid" *vs.* "control") were analyzed further.

Although it is often claimed that volume conduction is the main obstacle in interpreting EEG data in terms of brain connectivity, we have shown through modeling experiments that the values of the ISS are sensitive to the morpho-functional organization of the cortex rather than to the volume conduction and reference electrode (for relevant details, we address the reader to Kaplan et al. 2005; for further discussion see also Appendix A in Fingelkurts at al. 2006).


**Results**

**Local functional connectivity**

*EEG segment attributes*

Figure 1 displays the maps of opioid-induced changes in EEG segment attributes for alpha and beta activity (data averaged across all subjects). Corresponding data presented separately for five segment attributes (A, L, V, AR, and S, see Methods section).

Average amplitude (A) of EEG segments was significantly larger in opioid abusers ($P<0.05$–$P<0.001$ for different locations) when compared with healthy controls practically in all

EEG locations (Fig. 1). This was the case for both alpha and beta frequency bands. Average length (L) of EEG segments was longer also in opioid-abuse patients ($P<0.05$– $P<0.001$).
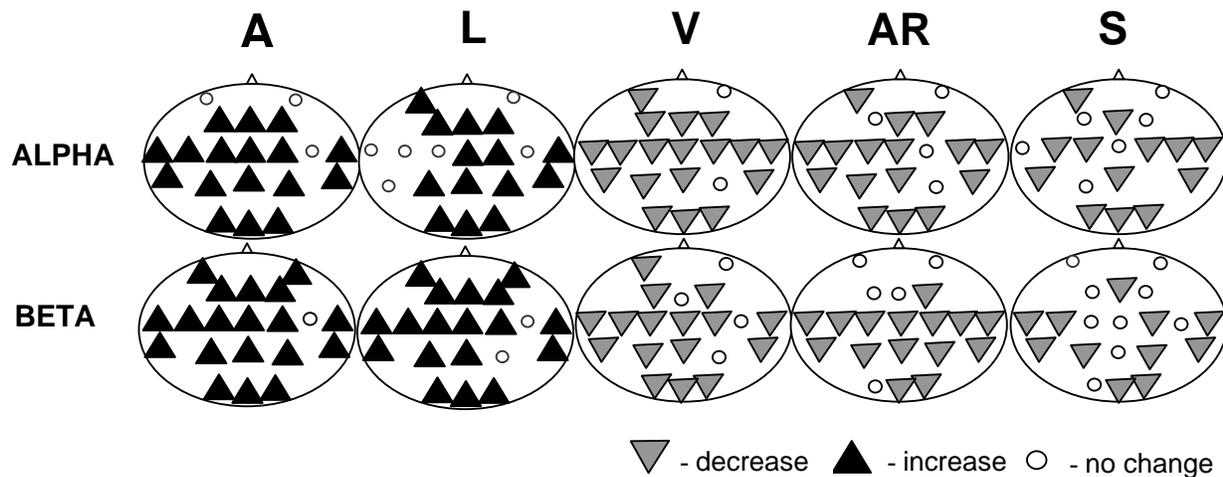


**Figure 1. The maps of opioid-induced statistically significant changes (after the Wilcoxon filtering; $P < 0.05$) in alpha and beta activity segment attributes for the chronic opioid-abuse patients.** Corresponding data presented separately for five EEG segment attributes, comparing the "opioid" patients with healthy "controls." EEG labels: first level of electrodes from the top – $F_7$, $F_8$; second – $F_3$, $F_Z$, $F_4$; third – $T_3$, $C_5$, $C_3$, $C_Z$, $C_4$, $C_6$, $T_4$; forth – $T_5$, $P_3$, $P_Z$, $P_4$, $T_6$; fifth – $O_1$, $O_Z$, $O_2$. $A$ – Average amplitude within segments ($\mu$V); $L$ – Average length of segments (msec); $V$ – Coefficient of amplitude variability within segments (%); $AR$ – Average amplitude relation among adjacent segments (%); $S$ – Average steepness among adjacent segments (estimated in the close area of RTP) (%).

However, in this case, left centro-parietal and right frontal areas for the alpha activity, and right centro-parietal areas for the beta activity, have not been affected (Fig. 1). The coefficient of amplitude variability (V) within EEG segments decreased significantly in opioid abusers ($P<0.05$–$P<0.01$) practically in all cortical areas, besides right prefrontal and central areas (for the alpha frequency band), and right frontal, central, and parietal areas (for the beta frequency band). Average amplitude relation (AR) among adjacent EEG segments decreased significantly in opioid-abuse patients ($P<0.05$) mainly in the posterior parts of the cortex. Both frequency bands exhibited very similar results (Fig. 1). Average steepness (S) among adjacent EEG segments decreased significantly in opioid abusers ($P<0.05$) in majority of cortical areas for alpha and beta activities (Fig. 1).

**EEG structural synchrony – remote functional connectivity**

*The number of structurally synchronized EEG pairs*

Figure 2 (top row) illustrates the number of SS EEGs registered from different cortical areas (estimated by ISS) in current opioid abusers and healthy control subjects. The number of SS EEG pairs was significantly lower ($P<0.05$–$P<0.01$ for different categories) in opioid-abuse patients than in healthy controls for most categories of functional connections. Generally, this was the case for both alpha and beta frequency bands. However, the strongest decrease in the number of functional connections for the alpha activity was observed in $\text{short}_{right/posterior}$ and $\text{long}_{posterior}$ categories, whereas $\text{short}_{right}$ and $\text{long}_{interhemisphere}$ categories exhibited the strongest decrease in the number of SS EEG pairs for the beta activity.

*The strength of the structurally synchronized EEG pairs*

The strength of structurally synchronized EEG pairs can be estimated by the values of ISS: the higher this value, the greater the strength. One important finding was the absence of negative values of ISS in all obtained combinations of EEG channels for alpha and beta frequency bands.

Figure 2 (bottom row) illustrates the mean values of ISS for the nine SS EEG pair categories in current opioid-abuse patients and healthy controls separately for alpha and beta EEG frequency bands. The ISS values were significantly lower in opioid abusers than in control subjects practically in all categories ($P<0.05$– $P<0.001$ for different categories). This dependence was observed in the alpha as well as in the beta frequency bands; however, the strongest decrease in the ISS values was found for the alpha activity in the $\text{short}_{right/posterior}$ and $\text{long}_{interhemisphere}$ categories of functional connections (Fig. 2, bottom row).
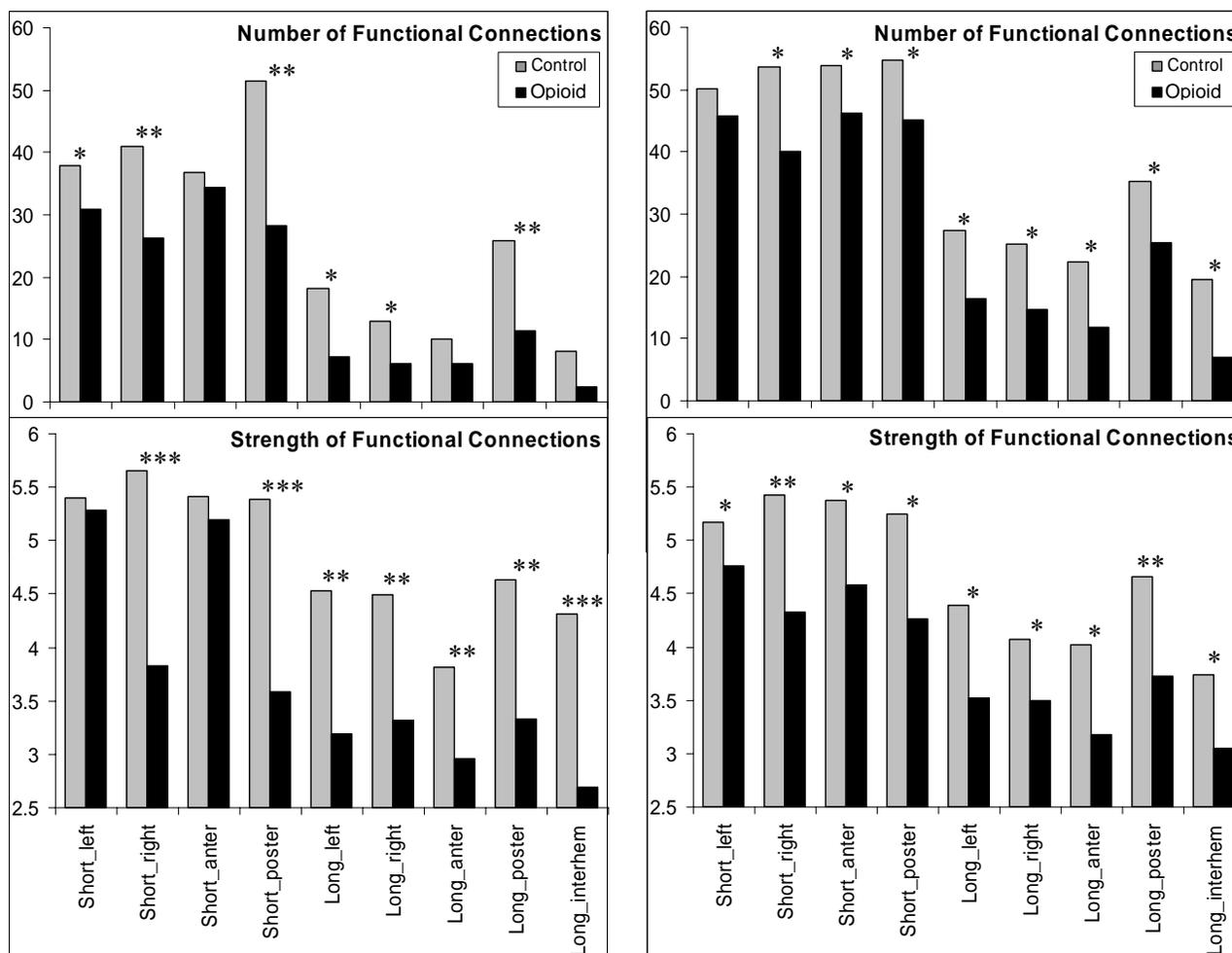
ALPHA                                    BETA

**Figure 2. The number (top row) and strength (bottom row) of structurally synchronized (SS) EEG pairs (indexed by the index of structural synchrony) in opioid-abuse patients and healthy subjects for eyes-closed condition separately for alpha and beta frequency bands.** The *X*-axis displays the labels of the categories for EEG pair connections. The *Y*-axis displays either the percentage from the maximum number of the EEG pair connections within each category (top row) or the average values of ISS for EEG pair connections within each category (bottom row). * – $P < 0.05$, ** – $P < 0.01$, *** – $P < 0.001$. *Control* – group of healthy subjects; *Opioid* – group of opioid-abuse patients.

*Relationships between duration of daily opioid abuse and EEG structural synchrony*

The duration of daily opioid abuse (DDOA) predicted negatively the number of long$_{posterior}$ connections (Pearson correlation = -0.89, $P<0.05$) and the strength of short$_{posterior}$ connections (Pearson correlation = -0.84, $P<0.05$) for alpha frequency band, and the strength of short$_{posterior}$ connections (Pearson correlation = -0.95, $P<0.05$) for beta frequency band.

*Topology of EEG structural synchrony*

Figure 3 displays the reliable statistically significant (*P*<0.05) ISS values mapped onto brain schemata as connecting lines between corresponding EEG sites in current opioid-abuse patients and control subjects for alpha and beta frequency bands.

Maps of synchronized cortical areas (indexed by ISS) differed in opioid-abuse and control groups. Indeed, the SS EEG pairs in control subjects were symmetrical for both alpha and beta frequency bands. In contrast, SS EEG pairs in opioid-abuse patients were asymmetric: they concentrated along left brain areas; and new frontal connection (F3-F7) appeared (Fig. 3). Additionally, there was one operational module (OM) with "3$^{rd}$ order of recruitment" in the right hemisphere of control subjects in beta frequency band (Fig. 3). This OM was absent in opioid abusers.

EEG/alpha          EEG/beta



Control          Opioid          Control          Opioid
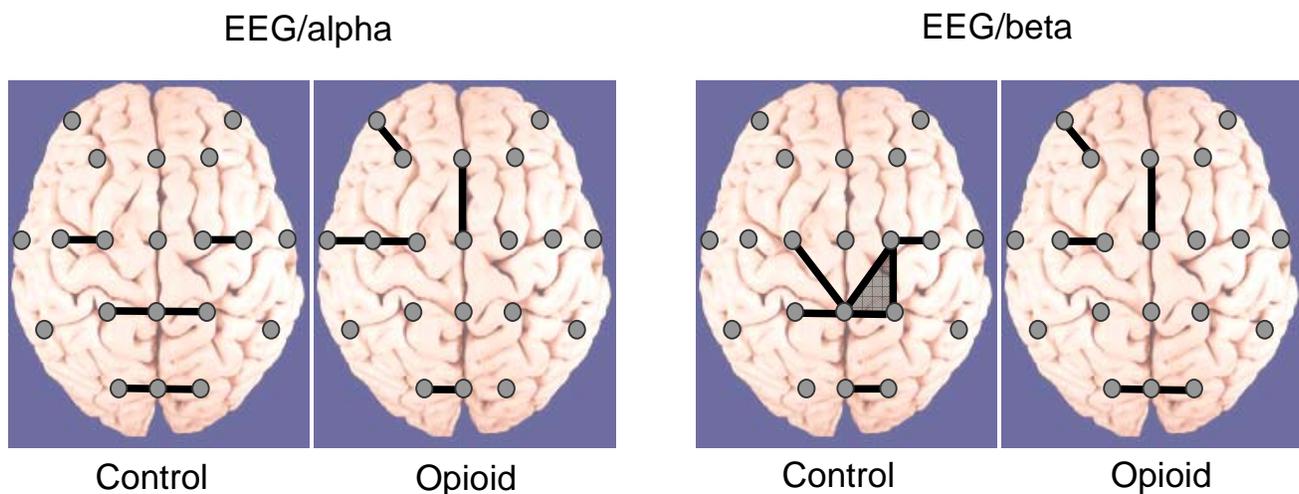
**Figure 3. The specific patterns of synchronized cortical areas (indexed by Index of Structural Synchrony – ISS) in chronic opioid-abuse patients and healthy subjects for the alpha and beta frequency bands.** The ISS values which occur more than in 85% of repetitions across all subjects are mapped onto schematic brain maps as connecting lines between the EEG channels involved. Grey areas indicate the operational module (OM). EEG labels: first level of electrodes from the top – $F_7$, $F_8$; second – $F_3$, $F_Z$, $F_4$; third – $T_3$, $C_5$, $C_3$, $C_Z$, $C_4$, $C_6$, $T_4$; forth – $T_5$, $P_3$, $P_Z$, $P_4$, $T_6$; fifth – $O_1$, $O_Z$, $O_2$. *Control* – group of healthy subjects; *Opioid* – group of opioid-abuse patients.

*Specificity of opioid-induced changes in the functional connectivity*

Considering that several patients besides opioid dependence met also DSM-IV criteria for benzodiazepine dependence, all patients were assigned to two analysis subgroups: "pure" opioid-dependent group and opioid+benzodiazepine-dependent group. Correlation analysis (Pearson correlations) between the number/strength of functional connections in these subgroups revealed very high values (0.8-1, *P*<0.001) for different categories of connections, thus conforming that

opioid effects which have been observed in the previous sections were indeed dominant and characteristic for all patients.

**Discussion**

We found evidence that the brain local functional connectivity increases and brain remote functional connectivity decreases in patients with opioid dependence (compared with healthy controls). Considering very high correlations between the number/strength of functional connections in opioid-dependent and opioid+benzodiazepine-dependent subgroups of patients, the findings presented in this article are determined mainly by the opioid dependence.

*Local functional connectivity*

We found in the present study that the individual attributes of neuronal assemblies were sensitive to the longitudinal opioid influence (Fig. 1). For both alpha- and beta-generated neuronal assemblies, it was observed that they were characterized by a total increase in their size (*A* attribute) and functional life span (*L* attribute). Simultaneously, these neuronal assemblies were more stable (*V* attribute) in the opioid abusers when compared with healthy controls. Such stability was accompanied by a very low speed (*S* attribute) of the functional elimination of some neurons from the large neuronal assemblies (*AR* attribute), probably indicating the process of compensation for the larger size of neuronal assemblies in these opioid abusers. These findings may be tentatively linked to the fact that opioids increase cell firing at the level of the cell bodies (Nutt 1996). Such elevated firing of neurons in its turn might result in the increase synchronization among neighboring neurons and the formation of large neuronal assemblies (Grün et al. 2002) in the brain of opioid-abuse patients.

Thus, obtained findings on the dynamic of neuronal assembly attributes indicated that the acute use of opioids (together with the longitudinal opioid usage history) results in the increase of local functional connectivity; and this was reflected in the formation of large and stable neuronal assemblies. Therefore, we may speculate that the brain of opioid dependent patients cannot reach a proper (for the healthy brain) resting state where individual brain areas, besides expressing their own functioning, are also heavily involved in a collective activity (Livanov 1984; Fingelkurts et al. 2003). Thus, such "normal" resting brain state is alien to the neuron-cognitive profile of "addicted" resting brain.

*Remote functional connectivity*

We found that the number and strength of remote functional connections among different cortical areas (estimated by an index of EEG structural synchrony, ISS) was significantly lower in opioid abusers than in healthy controls for most categories of functional connections (Fig. 2). This dependence was observed in the alpha as well as in the beta frequency bands. Such disruption in the functional synchrony among neuronal assemblies within distributed cortical areas is consistent with cortex processes disorganization syndrome (suggested by Bressler 2003). Most likely, the disrupted brain remote functional connectivity observed in the present study may constitute the candidate mechanism for a well documented pattern of impairment in addicts that expresses the lack of integration of different cognitive functions for effective problem solving, deficits in abstract concept formation, set-maintenance, set-shifting, behavioral control, and problems in the regulation of affect and behavior (Miller 1990; Ornstein et al. 2000; Davis et al. 2002). From this perspective then, disorganization can be viewed as a *disorder of the metastable balance* between large-scale integration (remote functional connectivity) and independent processing (local functional connectivity) in the cortex of chronic opioid addicts in favor of independent processing (recall the increased volume, stability, and life-span of local neuronal assemblies). However, the open question is whether the same trade-off (increased local and decreased remote functional connectivity) would be encountered in a study involving some cognitive task rather than just a resting state.

At the same time, the longitudinal opioid effects on remote cortex functional connectivity appeared to be stronger at the right hemisphere (short$_{right}$ connections) and posterior section of the cortex (short$_{posterior}$ connections). The number and strength of functional connections in these cortex sections decreased the most (Fig. 2). Such pattern of functional connectivity was especially pronounced for the alpha frequency band. Higher sensitivity of the right hemisphere to adverse chronic opioid effects in comparison to the left hemisphere has been already reported earlier (Davydov and Polunina 2004). Speculatively, these studies together may point to a more severe right brain dysfunction in opioid addicts' population. The role of posterior cortex areas needs to be addressed further.

*Relationship between DDOA and the number/strength of functional connections*

It has been shown that long-chronic opioid addicts have more impaired cognitive functioning than addicts of shorter duration (Cipolli and Galliani 1990). In this context we may expect to observe a stronger decrease in the number and strength of functional connections as a function of duration of daily opioid abuse (DDOA). If such significant dependence would exist,

then it will give serious evidence to suppose *adverse longitudinal opioid effect* as the underlying cause of brain functional connectivity changes in chronic opioid addicts. Our data demonstrated that some of the functional connections have indeed had a predictive force towards the DDOA: The less was the number of $long_{posterior}$ and strength of $short_{posterior}$ functional connections for the alpha frequency band, and the strength of $short_{posterior}$ functional connections for the beta frequency band, the more was DDOA value. In other words, the more years subject uses opioids daily, the more posterior section (low central, parietal, low temporal, and occipital areas) of the cortex becomes functionally disintegrated. Hence, these data give grounds to suppose that longitudinal opioid usage impairs especially remote functional connectivity in the posterior cortex section. Areas located in this cortex part suggested to be responsible for a number of important functions (Damasio 2000): perception, differentiation, and somatosensory functions (roughly $P_{3/z/4}$), memory functions and autobiographical records (roughly $T_{5/6}$), and visual perception (roughly $O_{1/2}$). We may speculate that the absence of appropriate relations between all these functions in opioid-abuse patients lead them to specific actions such as those aimed at drug-seeking and drug-taking behavior that characterize addiction (Robinson and Berridge 2000); and as the ravages of addiction's deepen, a progressive degradation of functional interrelations between different cortex areas responsible for those cognitive functions take place.

Generally, the significant statistical relationship between functional connectivity and opioid abusing duration gives serious ground to reject possible influence of comorbid psychiatric conditions (e.g., antisocial, obsessive-compulsive, paranoid, borderline, and personality disorders) or some other premorbid brain dysfunctions (Kaplan and Sadock 1994) and the effects of other psychotropic drugs abused throughout life.

*Topological aspects of functional connectivity in opioid addicts*

The most representative and stable topological combinations of functionally synchronized areas were located in the left frontal, central-temporal, and occipital cortex poles (Fig. 3). The fact that new frontal and midline functional connections appeared in the opioid patients' group which were absent in the healthy subjects is congruent with predictions that activity in these areas is determined mostly by the activity of limbic and cingulated regions (Mesulam 1986); and these neuronal systems seem to precisely support the reward-related behavior (Sell et al. 1999). Central-temporal areas have been proposed as memory integration cortices (Mesulam 1985), and in the present context, the functional connections among these regions may also reflect emotional learning / conditioning experienced by opioid addicts (Volkow et al. 2004).

*Alpha and beta synchronicity profiles*

Despite basic differences in their physiological reactivity and cognitive meaning (Basar et al. 2004), alpha and beta oscillations in opioid abusers showed large similarities in their profiles of local and remote functional connectivity. The modern view on cortex oscillations states that alpha frequency range is quite heterogeneous, whereas the slow alpha (~7-10 Hz) is mostly subcortical and upper alpha (~11-13Hz) is mostly cortical (Klimesch 1999). Beta frequency band is more homogeneous and considered to be exclusively cortical (Jensen et al. 2005). Since we found that synchronicity profiles have been very similar for alpha and beta frequency ranges, we propose that it was mostly the upper alpha rhythm that dominated the alpha frequency range in opioid addicts participated in the present study. This supposition was further supported by the short-term spectral patterns obtained from the same subjects (Fingelkurts et al. 2006b). In that study, it was demonstrated that EEG of chronic opioid-abuse patients was indeed dominated by the short-term spectral patterns with 9-13 Hz (additionally to the beta activity).

In conclusion, it is worth to note that the data about local and remote functional connectivity obtained in the present study (opioid addiction) have had exactly the opposite profile when compared with analogous data for bensodiazepines (GABA agonists), whereas we observed a decrease in local and an increase in remote cortex functional connectivity (Fingelkurts et al. 2004). Based on these findings, and considering a new strategy for treatment of brain disorders (Fingelkurts et al. 2005b), we may speculate that appropriate dosage of drugs that enhance GABA$_{ergic}$ systems in the brain may normalize the local and remote brain functional connectivity specifically in opioid addicts, and, therefore, may be considered as a possible new treatment for the opioid dependence. Indeed, GABA-enhancing drugs have been already shown some promising results in this context at least in animal studies (for the review, see Volkow et al. 2004).

## Acknowledgments

**References**

American Psychiatric Association (1994) Diagnostic and Statistical Manual of Mental Disorders – 4th edn. American Psychiatric Association: Washington, DC

Basar E, Özgören M, Karakas S, Basar-Eroglu C (2004) Super-synergy in the brain: the grandmother percept is manifested by multiple oscillations. Int J Bifurcat Chaos 14:453-491

Baxter MG, Parker A, Lindner CC, Izquierdo AD, Murray EA (2000) Control of response selection by reinforcer value requires interaction of amygdala and orbital prefrontal cortex. J Neurosci 20:4311–4319

Breakspear M, Terry JR (2002) Topographic organization of nonlinear interdependence in multichannel human EEG. NeuroImage 16:822–835

Bressler SL (2003) Cortical coordination dynamics and the disorganization syndrome in schizophrenia. Neuropsychopharmacology 28:S35–S39

Cipolli C, Galliani I (1990) Addiction time and value of Z indicators in Rorschachs of heroin users. Percept Mot Skills 70:1105-1106

Cook IA, O'Hara R, Uijtdehaage SH, Mandelkern M, Leuchter AF (1998) Assessing the accuracy of topographic EEG mapping for determining local brain function. Electroencephalogr Clin Neurophysiol 107:408-414

Damasio AR (2000) The feeling of what happens. Body, emotion and the making of consciousness. Vintage: London

Davis PE, Liddiard H, McMillan TM (2002) Neuropsychological deficits and opiate abuse. Drug Alcohol Depend 67:105–108

Davydov DM, Polunina AG (2004) Heroin abusers' performance on the Tower of London Test relates to the baseline EEG alpha2 mean frequency shifts. Prog Neuropsychopharmacol Biol Psychiatry 28:1143-1152

deCharms RC, Merzenich MM (1996) Primary cortical representation of sounds by the coordination of action-potential timing. Nature 381:610–613

Fingelkurts AnA, Fingelkurts AlA (2001) Operational architectonics of the human brain biopotential field: Towards solving the mind-brain problem. Brain Mind 2:261-296 Url: http://www.bm-science.com/team/art18.pdf

Fingelkurts AnA, Fingelkurts AlA (2003) Operational architectonics of perception and cognition: A principle of self-organized metastable brain states. VI Parmenides Workshop, Institute of Medical Psychology. April 5-10, Elba/Italy (invited full-text contribution). Url: http://www.bm-science.com/team/art24.pdf

Fingelkurts AnA, Fingelkurts AlA (2004) Making complexity simpler: Multivariability and metastability in the brain. Int J Neurosci 114:843-862

Fingelkurts AnA, Fingelkurts AlA (2005) Mapping of the brain operational architectonics. In: Chen FJ (ed) Focus on Brain Mapping Research. Chapter 2. Nova Science Publishers, Inc, pp. 59-98. Url: http://www.bm-science.com/team/chapt3.pdf

Fingelkurts AnA, Fingelkurts AlA, Kähkönen S (2005a) Functional connectivity in the brain – is it an elusive concept? Neurosci Biobehav Rev 28:827-836

Fingelkurts AnA, Fingelkurts AlA, Kähkönen S (2005b) New Perspectives in Pharmaco-Electroencephalography. Prog Neuropsychopharmacol Biol Psychiatry 29:193-199

Fingelkurts AlA, Fingelkurts AnA, Krause CM, Kaplan AYa (2003) Systematic rules underlying spectral pattern variability: Experimental results and a review of the evidences. Int J Neurosci 113:1447-1473

Fingelkurts AnA, Fingelkurts AlA, Kivisaari R, Pekkonen E, Ilmoniemi RJ, Kähkönen SA (2004) Local and remote functional connectivity of neocortex under the inhibition influence. Neuroimage 22:1390-1406

Fingelkurts AnA, Fingelkurts AlA, Rytsälä H, Suominen K, Isometsä E, Kähkönen S (2006a) Impaired functional connectivity at EEG alpha and theta frequency bands in major depression. Hum Brain Map DOI: 10.1002/hbm.20275

Fingelkurts AlA, Fingelkurts AnA, Kivisaari R, Autti T, Borisov S, Puuskari V, Jokela O, Kähkönen S (2006b) Reorganization of the composition of brain oscillations and their temporal characteristics in opioid dependent patients. Prog Neuropsychopharmacol Biol Psychiatry (in press).

First MB, Spitzer RL, Gibbon M, Williams JBW, Benjamin L (1994a) Structured clinical interview for DSM-IV axis II personality disorders – Version 2.0. New York State Psychiatric Institute: New York

First MB, Spitzer RL, Gibbon M, Williams JBW (1994b) Structured clinical interview for DSM-IV axis I disorders – Version 2.0. New York State Psychiatric Institute: New York

Franken IH, Stam CJ, Hendriks VM, van den Brink W (2004) Electroencephalographic power and coherence analyses suggest altered brain function in abstinent male heroin-dependent patients. Neuropsychobiology 49:105-110

Freeman WJ (2000) Brains create macroscopic order from microscopic disorder by neurodynamics in perception – Chapter 7. In: Århem P, Blomberg C, Liljenström H (eds) Disorder versus order in brain function: Essays in theoretical neurobiology. World Scientific Publishing Co: Singapore, pp 205-219

Freeman WJ (2003) Evidence from human scalp electroencephalograms of global chaotic itinerancy. Chaos 13:1067-1077

Freeman WJ, Kozma R, Werbos PJ (2001) Biocomplexity: Adaptive behavior in complex stochastic dynamical systems. Biosystems 59:109-123

Goldstein RZ, Volkow ND (2002) Drug addiction and its underlying neurobiological basis: neuroimaging evidence for the involvement of the frontal cortex. Am J Psychiatry 159:1642-1652

Gossop M (1990) The development of short opiate withdrawal scale (SOWS). Addict Behav 15:487–490

Grün S, Diesmann M, Aertsen A (2002) Unitary events in multiple single-neuron spiking activity: I. Detection and significance, II. Nonstationary data. Neural Comput 14:43-119

Hyman SE, Malenka RC (2001) Addiction and the brain: the neurobiology of compulsion and its persistence. Nature Rev Neurosci 2:695-703

Hyvärinen A, Karhunen J, Oja E (2001) Independent Component Analysis. John Wiley & Sons.

Jensen O, Goel P, Kopell N, Pohja M, Hari R, Ermentrout B (2005) On the human sensorimotor-cortex beta rhythm: Sources and modeling. Neuroimage 26:347-355

John ER (2001) A field theory of consciousness. Conscious Cogn 9:184-213

Joyce CA, Gorodnitsky IF, Kutas M (2004) Automatic removal of eye movement and blink artifacts from EEG data using blind component separation. Psychophysiology 41:313-325

Kaplan AYa (1998) Nonstationary EEG: methodological and experimental analysis. Usp. Physiol. Nayk (Success in Physiological Sciences) 29:35–55 (in Russian)

Kaplan AYa, Shishkin SL (2000) Application of the change-point analysis to the investigation of the brain's electrical activity – Chapter 7. In: Brodsky BE, Darkhovsky BS (eds) Nonparametric statistical diagnosis: problems and methods. Kluwer Acad Publ: Dordrecht, the Netherlands, pp 333-388

Kaplan AYa, Borisov SV (2003) Dynamic properties of segmental characteristics of EEG alpha activity in rest conditions and during cognitive load. Zh. Vyssh. Nerv. Deiat Im. IP Pavlova (IP Pavlov J. of Higher Nervous Activity) 53:22-32 (in Russian)

Kaplan AYa, Fingelkurts AnA, Fingelkurts AlA, Darkhovsky BS (1997) Topological mapping of the sharp reorganization synchrony in the multichannel EEG. Am J END Technol 37:265-275

Kaplan AYa, Borisov SV, Shishkin SL, Ermolayev VA (2002) The analysis of segmental structure of EEG alpha activity in humans. Fiziol Zh Im IM Sechenova (Physiology J IM Sechenov) 88:432-442 (in Russian)

Kaplan AYa, Fingelkurts AnA, Fingelkurts AlA, Borisov SV, Darkhovsky BS (2005) Nonstationary nature of the brain activity as revealed by EEG/MEG: Methodological, practical and conceptual challenges. Signal Process 85:2190-2212

Kaplan HI, Sadock BJ (1994) Synopsis of Psychiatry: Behavioural Sciences. Clinical Psychiatry – 7th edn. Williams & Wilkins: Baltimore MD

Kelso JAS (1995) Review of Dynamic Patterns: The Self-Organization of Brain and Behavior. MIT Press: Cambridge, MA

Klimesch W, Schack B, Sauseng P (2005) The functional significance of theta and upper alpha oscillations. Exp Psychol 52:99-108

Klimesch W (1999) Event-related band power changes and memory performance. Event-related desynchronization and related oscillatory phenomena of the brain. In: Pfurtscheller G, Lopez da Silva FH (eds) Handbook of electroencephalography and clinical neurophysiology – Vol. 6, Elsevier: Amsterdam, pp 151-178

Koenig T, Prichep L, Lehmann D, Sosa PV, Braeker E, Kleinlogel H, Isenhart R, John ER (2002) Millisecond by millisecond, year by year: normative EEG microstates and developmental stages. Neuroimage 16:41-48

Livanov MN (1984) Electroencephalogram rhythms and their functional significance. Zh Vyssh Nerv Deiat Im IP Pavlova (IP Pavlov J of Higher Nervous Activity) 34:613-626 (in Russian)

Mesulam M (1985) Principles of Behavioral Neurology. FA Davis: Philadelphia

Mesulam M (1986) Frontal cortex and behaviour. Ann Neurol 19:320-324

Miller L (1990) Neuropsychodynamics of alcoholism and addiction: personality, psychopathology, and cognitive style. J Subst Abuse Treat 7:31-49

Nutt DJ (1996) Addiction: brain mechanisms and their treatment implications. The Lancet 347:31-36

Noel BM, Gratton A (1995) Electrochemical evidence of increased dopamine transmission in prefrontal cortex and nucleus acumbens elicited by ventral tegmental μ-opioid receptor activation in freely behaving rats. Synapse 21:110-122

Nunez PL (2000) Toward a quantitative description of large-scale neocortical dynamic function and EEG. Behav Brain Sci 23:371-437

Ornstein TJ, Iddon JL, Baldacchino AM, Sahakian BJ, London M, Everitt BJ, Robbins TW (2000) Profiles of cognitive dysfunction in chronic amphetamine and heroin abusers. Neuropsychopharmacology 23:113-126

Polunina AG, Davydov DM (2004) EEG spectral power and mean frequencies in early heroin abstinence. Prog Neuropsychopharmacol Biol Psychiatry 28:73– 82

Rappelsberger P, Petsche H (1988) Probability mapping: power and coherence analysis of cognitive processes. Brain Topogr 1:46-54

Robinson TE, Berridge KC (2000) The psychology and neurobiology of addiction: an incentive-sensitization view. Addiction 95:S91–S117

Robinson TE, Gorny G, Savage VR, Kolb B (2002) Widespread but regionally specific effects of experimenter- versus self-administered morphine on dendritic spines in the nucleus accumbens, hippocampus, and neocortex of adult rats. Synapse 46:271–279

Sell LA, Morris J, Bearn J, Frackowiak RSJ, Friston KJ, Dolan RJ (1999) Activation of reward circuitry in human opiate addicts. Eur J Neurosci 11:1042-1048

Singer W, Gray CM (1995) Visual feature integration and the temporal correlation hypothesis. Annu Rev Neurosci 18:555–586

Stam CJ (2005) Nonlinear dynamical analysis of EEG and MEG: Review of an emerging field. Clin Neurophysiol 116:2266–2301

Truccolo WA, Ding M, Knuth KH, Nakamura R, Bressler S (2002) Trial-to-trial variability of cortical evoked responses: implications for analysis of functional connectivity. Clin Neurophysiol 113:206-226

Varela FJ (1995) Resonant cell assemblies: A new approach to cognitive functions and neuronal synchrony. Biol Res 28:81-95

Volkow ND, Fowler JS, Wang G-J (2004) The addicted human brain viewed in the light of imaging studies: brain circuits and treatment strategies. Neuropharmacology 47:3-13

von Stein AV, Rappelsberger P, Sarnthein J, Petsche H (1999) Synchronization between temporal and parietal cortex during multimodal object processing in man. Cereb Cortex 9:137-150

Wechsler D (1993) Wechsler Adult Intelligence Scale-Revised (WAIS-R). Finnish version. Psykologien Kustannus: Helsinki, Finland

Weiss S, Rappelsberger P (2000) Long-range EEG synchronization during word encoding correlates with successful memory performance. Cog Brain Res 9:299-312

White NM (1989) A functional hypothesis concerning the striatal matrix and patches: mediation of S-R memory and reward. Life Sci 45:1943–1957