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Methadone Restores Local and Remote EEG Functional Connectivity in Opioid-Dependent Patients

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Abstract

Currently it has been proposed that normal brain function is critically dependent upon a dynamical balance between functions of local neuronal assemblies and global integrative processes. A loss of such metastable balance in favor of either independent or hyper-ordered processing is considered as a reflection of a brain disease. It has been shown that opioid dependence can be characterized as a disease of brain metastable balance, whereas local functional connectivity (synchronicity within neuronal assemblies) increased and remote functional connectivity (synchronicity between neuronal assemblies) decreased. Since methadone may be used as a maintenance treatment for opioid dependent patients, the aim of this research was to study how methadone would influence the temporal and metastable cortical organization through the measures of local and remote EEG functional connectivity in six opioid-dependent patients who manage to complete at least six-month methadone treatment. The present study demonstrated that average parameters of temporal and metastable organization of the cortical dynamics (indexed by local and remote EEG functional connectivity) in such opioid-dependent patients did not differ from normal values of healthy subjects. We interpret these findings as a capability of the methadone to restore a normal temporal and metastable structure of brain activity in opioid-dependent patients after many months of methadone treatment. To our knowledge, present preliminary study is the first where the influence of methadone on temporal and metastable structure of EEG activity was demonstrated.

Keywords: addiction; brain interactions; connectivity; cortex; disease; health; metastability; metastable states; neurobiology; opiate; opioid dependence; pathology; structural synchrony; synchronization

Abbreviations:

Average Amplitude (A)

Average Amplitude Relation (AR)

Average Length (L)

Average Steepness (S)

Coefficient of Amplitude Variability (V)

Diagnostic and Statistical Manual of Mental Disorders (DSM)

Electroencephalography (EEG)

Independent Component Analysis (ICA)

Index of Structural Synchrony (ISS)

Operational Modules (OM)

Rapid Transition Processes (RTP)

Structural Synchrony (SS)

Structured Clinical Interviews (SCID)

Introduction

Modern neuroscience considers normal brain function to be the product of a large-scale network of coupled neuronal assemblies exhibiting transient and inherently metastable dynamics (Kelso, 1995; Friston, 1997; Fingelkurts & Fingelkurts, 2001) due to the interplay of functional integration on the one hand and functional segregation on the other (for the reviews, see Bressler & Kelso, 2001; Stam et al., 2003; Fingelkurts & Fingelkurts, 2004). In this context, it has been proposed that normal brain function is critically dependent upon a dynamical balance between functions of local, specialized neuronal assemblies and global integrative processes (Tononi et al., 1994; Fingelkurts et al., 2005a). A loss of such metastable balance in favor of either independent or hyper-ordered processing is considered as a reflection of a brain disease (Bressler, 2003), since such processes are not efficient in the healthy brain (Friston, 2000).

In our previous work we have shown that opioid dependence can be characterized as a disease of brain metastable balance, whereas local functional connectivity (synchronicity within neuronal assemblies) increased and remote functional connectivity (synchronicity between neuronal assemblies) decreased (Fingelkurts et al., 2006). We have suggested further, that one way to achieve a successful treatment of such diseases, is to use psychoactive drugs that would act through normalization of brain metastable and temporal balance (Fingelkurts et al., 2005a).

The methadone maintenance treatment of opioid dependence has increasingly become a standard practice in many countries (Maremmani & Reisinger, 1995). By acting on the same opioid receptors as opioids, methadone prevents the physical symptoms of withdrawal that occur when opioids are stopped. Traditionally, this fact is interpreted as a preventive action of methadone for the onset of physical cravings and therefore leads to reducing use of opioids (Simpson & Sells, 1982; Ball & Ross, 1991). However, there is no any knowledge whatsoever about methadone capacity to influence the temporal and metastable organization of brain processes, and thus having the possibility to normalize (through these processes) the expression of the disease at the behavioral-psychophysiological level.

In this context, it is important to study a) how methadone maintenance would influence the temporal and metastable cortical organization in opioid-dependent patients and b) to reveal if methadone could actually restore the normal cortical metastable balance in such patients. Recent techniques for estimation of cortical temporal and metastable organization include EEG (and MEG) segmentation procedures and a measure of EEG structural synchrony between local segmental descriptions (Kaplan et al., 1997, 2005; Fingelkurts et al., 2005b). These approaches are very sensitive to drug effects on brain dynamics (Fingelkurts et al., 2004), and in contrast to

conventional methods allow revealing the (i) individual microstates of various types in accordance with the nature of quasi-stationary EEG segments (local functional synchrony); and (ii) the formation of metastable states (remote functional synchrony) between distant cortical areas (Fingelkurts & Fingelkurts, 2005). For the limitations of conventional methods of EEG analysis see Fingelkurts et al., 2005b. Additionally, considering that there is no published research on EEG functional connectivity under the methadone influence, the present preliminary study is of particular interest and importance.

This study has been designed as a final part of the longitudinal research program and was aimed to explore the capacity of methadone treatment to restore until normal values the local and remote functional cortical connectivity at alpha and beta frequency bands in the opioid-dependent patients.

Materials and methods

Subjects

The study included a total of six right-handed, opioid-dependent patients, three men and three women aged between 25 and 41 years of age (33 ± 5 years) and 14 right-handed controls, six men and eight women aged 33 ± 5 years. These 6 patients were those patients who were among the 22 opioid-dependent patients in acute state (aged 33 ± 5 years, 14 males) (Fingelkurts et al., 2006) and among 13 patients (aged 32 ± 5 years, 5 males) agreed to undergo a withdrawal (Fingelkurts et al., 2007a) and at last manage to complete at least six-month methadone treatment (11 ± 6 months). All patients were hospitalized 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 that made routine therapy impossible, and alcohol dependence. In the present study, additional exclusion criteria for both patients and controls were major head trauma and neurologic illness.

All patients had abused opioids for 5–26 years (11 ± 7 years). Self-reported daily dose was 0.5–1 g for i.v. street heroin and 4–16 mg for i.v. street 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 (3 patients), cannabis (3 patients),

and amphetamine (3 patients) when heroin was not available. However, street buprenorphine and heroin was 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) that afford detailed information according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (American Psychiatric Association, 1994). All patients met DSM-IV criteria for opioid dependence, while three 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 personality disorder, diagnosed in all except three patients, who nonetheless had some features of antisocial personality disorder. Patients also fulfilled criteria of other personality disorders, such as obsessive-compulsive, paranoid, borderline, narcissistic, schizoid, passive-aggressive, dependent, and depressive personality disorders. 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. Controls did not fulfill any criteria for DSM-IV disorders on SCID I or II.

This study was carried out in accordance with the Declaration of Helsinki and was accepted by the Ethics Committees of Helsinki University Central Hospital. All the subjects studied gave informed written consent before enrolling in the study.

Trial design

Before methadone treatment starts, all patients had been abstinent for 12-15 days (Fingelkurts et al., 2007a). At the time of the EEG assessment, patients had been under the methadone support treatment at least six months (11 ± 6 months). During this period patients were visited frequently (i.e. twice per week) and unpredictably for the purpose of detecting relapse or continued abstinence via urine and breath screening. For the management of withdrawal symptoms in patients, methadone was used once in the morning in dosages 95-150 mg (depending on the patient). All patients have had blood test to trace the opioid (and other drugs, such as benzodiazepins) signs on the day of EEG admission and all had negative results.

Following electrode placement and instruments 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 comfortable position and to avoid movement. 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. Electric spontaneous brain activity 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).

EEG was recorded with an electrode cap according to the International 10/20 extended system and 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 Ω . 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). By the same procedure we can filter off a wide range of artifacts, improving the relative amount of any types of useful information in the signal (Cichocki et al., 2005). The ICA procedure is implemented as “The FastICA package for MATLAB” 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: “methadone” and “control.” Further data processing was performed separately for each 1-min portion of the signal. This increases the effective number of degrees of freedom and improves the statistical confidence in the results. Due to the technical requirements of the tools which 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, 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 during opioid addiction/abstinence were observed in alpha and beta ranges (for the review, see Polunina & Davydov, 2004).

Estimation of the local functional interrelations

Local functional interrelations were estimated in two stages. At the *first stage*, the adaptive level segmentation of local EEGs was performed. Each 1-min EEG was segmented using method of identification of rapid transition processes (RTP) in the EEG amplitude (*RTPseg* tool). For the detail description see Fingelkurts et al. (2007c). 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., 2003a,b). With this technique, the sequence of RTPs with statistically proven ($P < 0.05$, Student *t*-test) time coordinates has been determined for each channel of each 1-min EEG. The theoretical concepts behind this analysis are described elsewhere (Kaplan, 1998; Kaplan & Shishkin, 2000; Fingelkurts et al., 2005b; Fingelkurts & Fingelkurts, 2005).

At the *second stage*, after quasi-stationary segments (indexed by RTPs) were obtained, several characteristics (attributes) of segments (Kaplan & 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 (Fell et al., 2000; 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 & 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 & Borisov, 2003).

Estimation of the remote functional connectivity

Remote functional connectivity was estimated by calculation of the index of EEG structural synchrony. The index of structural synchrony (ISS) was estimated through synchronization of RTPs between different EEG channels (*RTPsyn* tool). Details can be found in Fingelkurts et al. (2007c). 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., 2005b). The ISS tends towards zero where there is no synchronization between the EEG segments and has positive or negative values where such synchronization exists. 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 (Kaplan et al., 1997; Fingelkurts & Fingelkurts, 2001, 2004, 2005).

Using pair-wise analysis, structural synchrony (SS) was identified in several channels (more than two). These are described as operational modules – OM (Fingelkurts & Fingelkurts, 2001, 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 index of structural synchrony (Fingelkurts & Fingelkurts, 2005). The number of cortical areas recruited in OM is described as “the order of areas recruitment.”

Statistics

1. For each condition (“methadone” 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 & Borisov, 2003; Fingelkurts et al., 2004, 2006, 2007a), the comparison of the same segment attributes between different group-conditions was performed using Wilcoxon *t*-test.
2. The differences in the number and strength of structurally synchronized (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 Rappelsberger, 1998; Weiss & Rappelsberger, 2000). All SS pair EEG patterns were divided into nine categories ($\text{short}_{\text{left/right}}$, $\text{short}_{\text{anterior/posterior}}$, $\text{long}_{\text{left/right}}$, $\text{long}_{\text{anterior/posterior}}$, $\text{long}_{\text{interhemispheric}}$) separately for alpha and beta frequency bands. Pairs of EEG electrodes which have one or more electrodes between the “members” of the pair were classified as long-range connections (according to Weiss & 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.
3. 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 & Petsche, 1988) in the SS maps. This problem is common to all studies which require multiple comparisons

between maps (Weiss & Rappelsberger, 2000; Razoumnikova, 2000). The comparisons that have been made should therefore be considered descriptive rather than confirmatory (Stein et al., 1999). To have valid results and to overcome the multiple comparisons problem (for justification, see Appendix in Fingelkurts et al., 2007c) as we have done in our previous work (Fingelkurts et al., 2003a,b, 2004, 2006, 2007a,c), 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 (“methadone” vs. “control”) were analyzed further.

4. Although it is often claimed that volume conduction is the main obstacle in interpreting EEG data in terms 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 methodological discussion, see appendixes in Fingelkurts et al., 2007c).

Results

EEG segment attributes – local functional connectivity

Table 1 presents the mean values of EEG segment attributes for all channels and subjects within ‘methadone’ and ‘control’ comparison groups. Corresponding data presented separately for five segment attributes (A, L, V, AR, and S, see Methods section). The target of the present article was the comparison of healthy controls and opioid-dependent patients after at least six-month of methadone treatment.

Average amplitude (A), average length (L), and the coefficient of amplitude variability (V) of EEG segments were identical in patients and healthy controls. Average amplitude relation (AR) and average steepness (S) among adjacent EEG segments were significantly smaller in methadone-treated patients than in healthy controls. This was the case for both alpha and beta frequency bands.

Table 1. Comparisons of EEG segment attributes (mean for all channels) in healthy controls and for the chronic opioid-dependent patients after 11 months (on average) of methadone-supported treatment.

Rhythm	Attribute	Control	Methadone	<i>P</i> (CxM)
ALPHA	A	80 (7.9)	80 (23)	n.s.
	L	31 (0.3)	31 (0.2)	n.s.
	V	61 (0.2)	61 (0.4)	n.s.
	AR	118 (1.0)	117 (1.4)	< 0.05
	S	117 (0.6)	116 (0.8)	< 0.05
BETA	A	49 (3.3)	52 (17)	n.s.
	L	26 (0.1)	26 (0.1)	n.s.
	V	67 (0.3)	67 (0.4)	n.s.
	AR	104 (1.6)	103 (1.5)	< 0.05
	S	97 (0.8)	96 (0.9)	< 0.05

Attribute, EEG segment attributes; Control, healthy control subjects; Methadone, methadone treatment opioid-dependent patients. Values: Mean (\pm StD). *A*, Average amplitude within segments (μ 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) (%). (CxM), comparison between control and methadone conditions.

Figure 1 presents the maps of methadone-induced changes in EEG segment attributes within separate EEG locations for alpha and beta activity (data averaged across all subjects).

Average amplitude (*A*) of EEG segments was significantly larger in methadone-treated patients ($P < 0.05$) in left frontal and right temporal cortical areas, and was significantly lower in the right parietal location when compared with healthy controls. This was the case for both alpha and beta frequency bands. Average length (*L*) of EEG segments was shorter in right central (alpha, beta bands), right parietal (beta band), and midline occipital (alpha band) cortex locations ($P < 0.05$) in patients as compared with controls.

The coefficient of amplitude variability (*V*) within EEG segments decreased significantly in methadone-supported patients ($P < 0.05$) in left prefrontal (alpha band) and left temporal (beta band) cortical areas, and increased ($P < 0.05$) in the right central location for both alpha and beta oscillations. Average amplitude relation (*AR*) among adjacent EEG segments decreased significantly in patients ($P < 0.05$) in many (mostly left) posterior EEG locations for alpha frequency band and in many left temporal-frontal cortex locations for the alpha frequency band.

However, right frontal area exhibited increase of AR values in methadone-supported patients (Fig. 1).

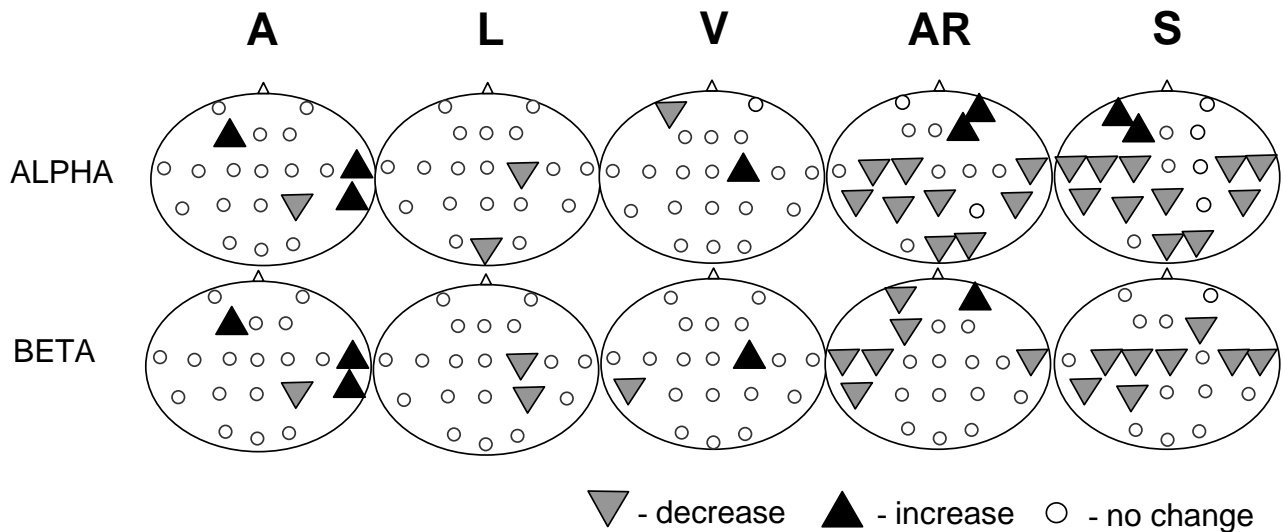


Figure 1. The maps of methadone-induced changes (after the Wilcoxon filtering; $P < 0.05$) in alpha and beta activity segment attributes for the opioid-dependent patients. Corresponding data presented separately for five EEG segment attributes, comparing the ‘methadone’ patients with healthy ‘controls.’ EEG labels: first level of electrodes from the top – F₇, F₈; second – F₃, F_Z, F₄; third – T₃, C₅, C₃, C_Z, C₄, C₆, T₄; forth – T₅, P₃, P_Z, P₄, T₆; fifth – O₁, O_Z, O₂. A, Average amplitude within segments (μ 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) (%).

Average steepness (S) among adjacent EEG segments decreased significantly in methadone-supported patients ($P < 0.05$) in many cortical areas along the central-temporal line for alpha and beta activities (Fig. 1). Exceptions were right frontal and prefrontal cortex areas (for alpha frequency band), which exhibited significant increase of this index in patients.

EEG structural synchrony – remote functional connectivity

The number and strength of functional connections in EEG pairs

Table 2 presents the mean values of number and strength of functional connections for all EEG pair combinations and all subjects within ‘control’ and ‘methadone’ comparison groups. Corresponding data presented separately for alpha and beta frequency bands.

The average values of number and strength of structurally synchronized (SS) EEGs (estimated by an index of structural synchrony, ISS) were nearly identical between healthy controls and methadone-supported patients. The difference was insignificant for both alpha and beta frequency bands.

Table 2. Comparisons of number and strength of functional connections (mean for all EEG pairs) in controls and for the opioid-dependent patients after 11 months (on average) of methadone-supported treatment.

	Control	Methadone	<i>P</i> (CxM)
ALPHA			
Number %	27 (12)	27.5 (10)	n.s.
Strength	4.8 (0.5)	5.1 (0.9)	n.s.
BETA			
Number %	36.2 (12)	35.7 (11)	n.s.
Strength	4.8 (0.5)	4.9 (0.6)	n.s.

Number, number of functional connections; Strength, strength of functional connections; Control, healthy control subjects; Methadone, methadone treatment opioid-dependent subjects. Values: Mean (\pm StD).

Figure 2 (top row) illustrates the number of structurally synchronized (SS) EEGs (grouped in different categories) in methadone-treated patients and in healthy control subjects. The number of SS EEG pairs was significantly lower ($P < 0.05$ – $P < 0.01$) for short_{posterior} category (alpha and beta bands), whereas it was significantly higher for long_{interhemisphere} category in patients than in healthy controls.

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

Figure 2 (bottom row) illustrates the mean values of ISS for the nine SS EEG pair categories in methadone-treated patients and healthy controls separately for alpha and beta EEG frequency bands. Only in short_{left/anterior/posterior} (for alpha band) and in short_{left/anterior} (for beta band) categories the ISS values were significantly higher in patients than in control subjects ($P < 0.05$). The significant decrease in the ISS values was found only for the long_{interhemisphere} category within the alpha frequency band (Fig. 2, bottom row).

Topology of EEG structural synchrony

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

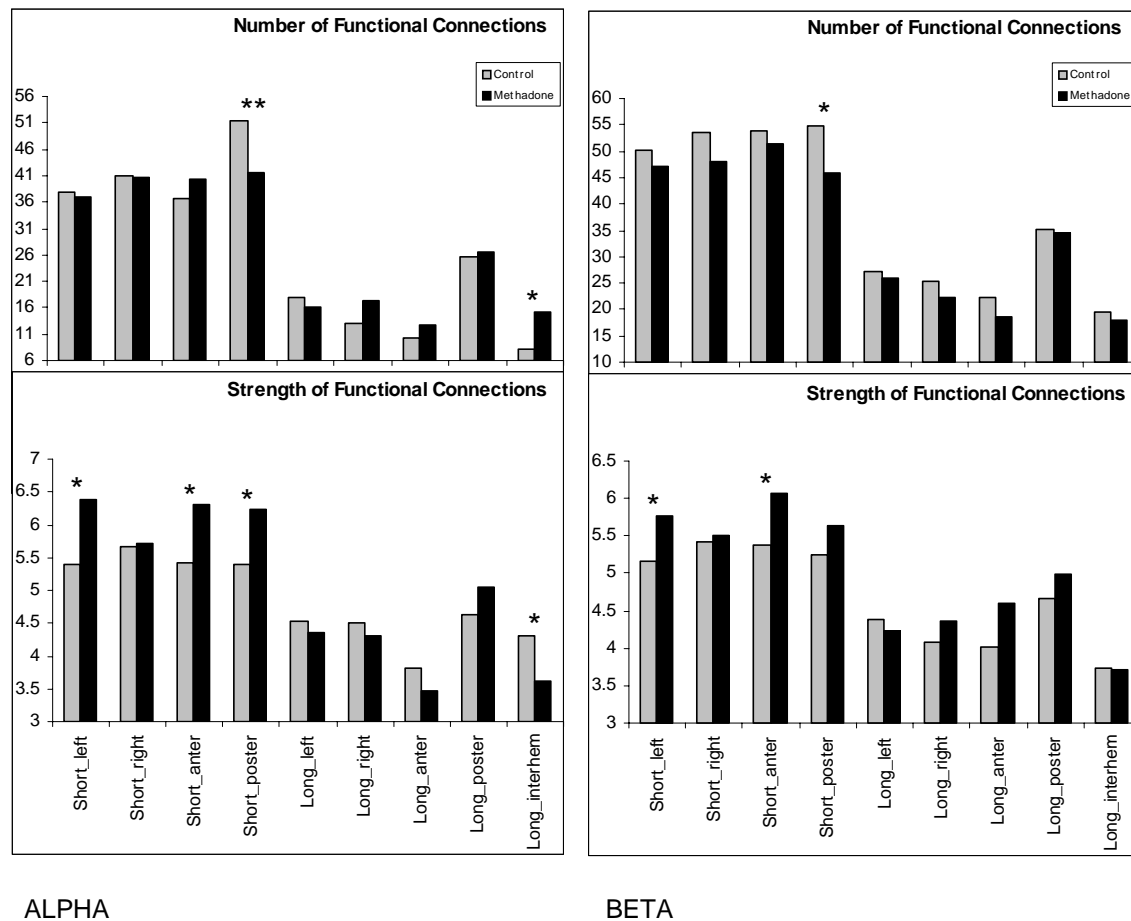


Figure 2. The number (top row) and strength (bottom row) of structurally synchronized (SS) EEG pairs (indexed by the Index of Structural Synchrony, ISS) in methadone-treated patients and healthy subjects separately for alpha and beta frequency bands. The X-axis displays the labels of the categories for 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). * – $P < 0.05$, ** – $P < 0.01$; *Control*, group of healthy subjects; *Methadone*, group of methadone-treated patients.

Maps of synchronized cortical areas (indexed by ISS) differed in patient and control groups. Indeed, the SS EEG pairs in control subjects were mostly symmetrical for both alpha and beta frequency bands. In contrast, SS EEG pairs in methadone-treated patients were asymmetric: they concentrated mostly within right occipital, parietal, central, and frontal cortex areas for the alpha activity, and within central-frontal section of the cortex for beta frequency band (Fig. 3).

There was one operational module (OM) with “3rd order of recruitment” in the right hemisphere of control subjects in the beta frequency band (Fig. 3). This OM was absent in the patients.

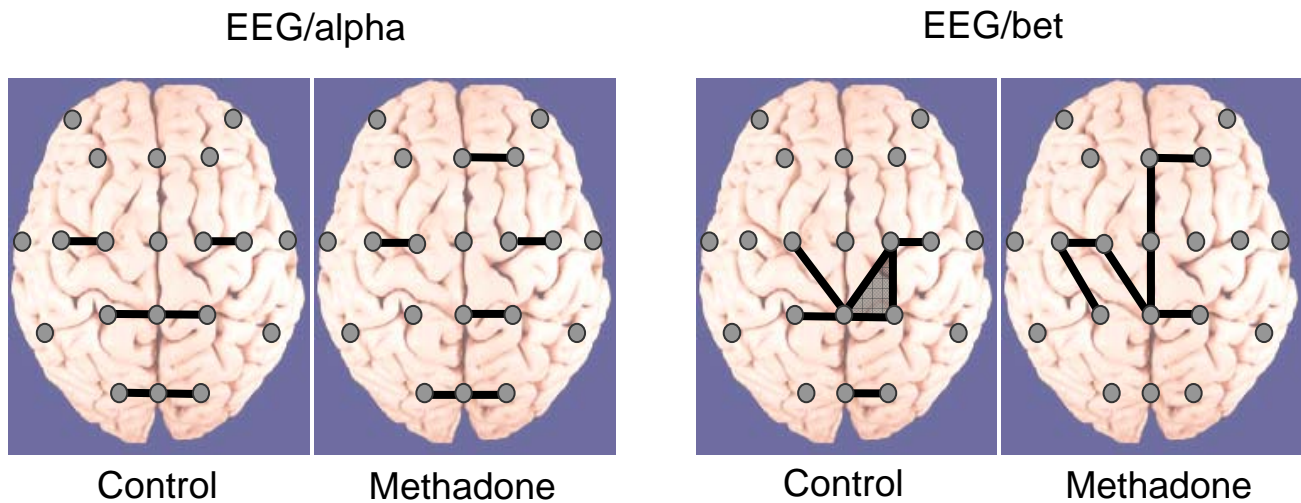


Figure 3. The specific patterns of synchronized cortical areas (indexed by the Index of Structural Synchrony, ISS) in methadone-treated patients and healthy subjects for the alpha and beta frequency bands. The ISS values which occur more than in 85% of repetitions across all subjects are mapped onto 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 – F₇, F₈; second – F₃, F_Z, F₄; third – T₃, C₅, C₃, C_Z, C₄, C₆, T₄; forth – T₅, P₃, P_Z, P₄, T₆; fifth – O₁, O_Z, O₂. *Control*, group of healthy subjects; *Methadone*, group of methadone-treated patients.

Discussion

Findings of the present study revealed that long-lasting methadone treatment can modify the temporal and metastable organization of the EEG in opioid-dependent patients, and that these methadone-induced changes either approach, or coincide completely with the normal parameters (healthy subjects) of cortical functional connectivity.

Methodological aspect

As stated in the Methods section this longitudinal study started with 22 opioid abusers (Fingelkurts et al., 2006), 13 of whom were studied during withdrawal (Fingelkurts et al., 2007a) and 6 of whom (current study) managed to keep on methadone maintenance for at least 6 months (on average 11 months). The results of current study indicated that there is little difference between the methadone-maintained patients and healthy volunteers. However, 6 patients participated in the current study may well have had more normal functional connectivity parameters at the most beginning compared with the other 16 patients who dropped out of the study; and that may have been the reason why these 6 patients managed to make it through to methadone treatment.

This is unlikely due to the following reasons: (1) 6 patients participated in the current study did not differ statistically significant in local and remote functional connectivity during the starting experimental phase (acute stage of abuse) from the remaining patients of the experimental group: Pearson correlation analysis between the parameters of local and remote functional connectivity in the current 6 patients and the rest of subgroup revealed very high values (0.78–1, $P < 0.001$). This means that current 6 patients had notable statistically significant differences in their local and remote functional connectivity from the control subjects (Fingelkurts et al., 2006). (2) These 6 patients also had the same personality disorders as the other patients of a larger group (compare with Table 1 in Fingelkurts et al., 2007b). Therefore, 6 patients participated in the current study did not differ in any initial characteristics (neither neurophysiologic, nor demographic) from the remaining 16 patients who were recruited by Helsinki University Central Hospital at the first experimental stage.

Figure 4 illustrates the dynamics of parameters of local and remote functional connectivity present in the EEG of opioid-dependent patients, from those currently with an opioid dependency, through withdrawal to the end of methadone treatment and compares them with analogous parameters of EEG of healthy subjects. As can be seen from this figure, all studied EEG parameters of opioid-dependent patients reached (or approached) the level of the healthy subjects after many months of methadone treatment.

Local functional connectivity

We found in the present study that in methadone maintaining patients the average attribute values of alpha- and beta-generated neuronal assemblies, such as the size (A), functional life span (L), and stability (V) did not differ from the values observed in the healthy subjects (Table 1). Even though the values of such attributes as the speed (S) of the recruitment and the exclusion of neurons (AR) from the neuronal assemblies were different from the normal subjects, these values have approached the analogous values of normal subjects, when compared with acute opioid (Fingelkurts et al., 2006) and abstinence (Fingelkurts et al., 2007a) conditions (see Fig. 4). Therefore, findings of the present and our previous (opioid addiction and abstinence) studies allow us to conclude that methadone treatment normalizes main parameters of the cortical neuronal assemblies which are reflected in the temporal characteristics of EEG signal; and which may constitute the neurobiological underpinnings of beneficial effects of methadone treatment (Ball & Ross, 1991).

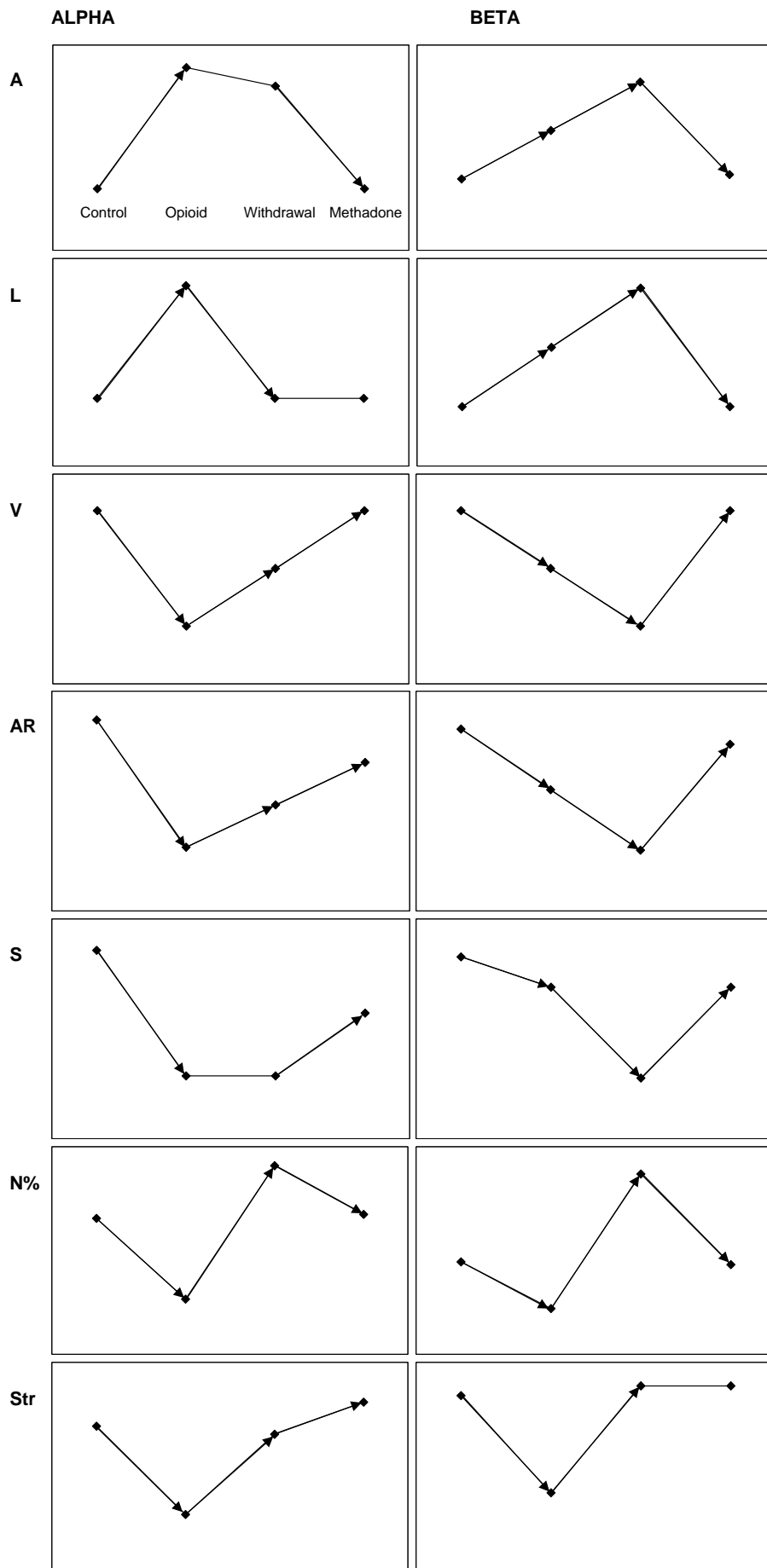


Figure 4. The scheme of the changes in the local (EEG segment attributes: *A*, *L*, *V*, *AR*, *S*) and remote (*N*, *Str*) functional connections for both alpha and beta frequency bands. The scheme scale is proportional to the real data. Data averaged across all subjects and all EEG channels/combinations of them. The experimental conditions are presented in chronological sequence: control (healthy subjects), current opioid dependency, withdrawal, and methadone maintenance treatment. Solid arrows indicate significant changes and solid lines mark insignificant changes. *A*, amplitude within segments (μV); *L*, length of segments (msec); *V*, Coefficient of amplitude variability within segments (%); *AR*, amplitude relation among adjacent segments (%); *S*, steepness among adjacent segments (estimated in the close area of RTP) (%); *N*, number of functional connections (%); *Str*, strength of functional connections.

However, in the methadone-treated patients, some individual cortical locations exhibited small but significant differences in the EEG segment attributes from the values of analogous attributes of healthy EEG. Such EEG segment attributes reflect different aspects of neuronal assemblies' dynamics (Kaplan & Borisov, 2003; Fingelkurts et al., 2004, 2006). We have observed that the size (*A*), life span (*L*), and stability (*V*) of neuronal assemblies, and especially the speed (*S*) of the process of functional elimination (*AR*) of neurons from the neuronal assemblies differed for some cortical locations between patients and normal controls (Fig. 1). These findings indicate that methadone-treated patients still had some significant differences in the characteristics of local functional connectivity when compared with healthy controls.

Remote functional connectivity

Findings of the present study indicated that on average remote functional connectivity of brain processes in distributed neuronal networks at different cortical areas (estimated by the index of EEG structural synchrony, ISS) was almost identical in patients and in healthy controls (Table 2). This was observed in the alpha as well as in the beta frequency bands. These findings together with our previous study of opioid dependence (Fingelkurts et al., 2006) and withdrawal (Fingelkurts et al. 2007a) conditions might be interpreted as evidence that methadone treatment in opioid-dependent patients have restored the average (total) parameters of remote functional connectivity until the normal levels (see Fig. 4) and consequently have normalized the metastable organization of the cortex (for a discussion, see Fingelkurts & Fingelkurts, 2004). This conclusion is consistent with current theoretical view that normal brain function is the product of a large-scale network of coupled neuronal assemblies exhibiting transient and inherently metastable dynamics (Kelso, 1995; Friston, 1997; Breakspear & Terry, 2002; Fingelkurts et al., 2005a; Fingelkurts & Fingelkurts, 2005).

However, some categories of cortical functional connections still exhibited significant differences in methadone-treated patients when compared with healthy subjects (Fig. 2). The number of short-posterior functional connections was diminished (for both frequency bands), whereas the strength of short-left (both frequencies), short-anterior (both frequencies), and short-posterior (alpha band) functional connections increased in the patients. This, may eventually explain, why in several studies it has been shown that methadone treatment was associated with a number of altered brain and cognitive parameters when compared with healthy subjects (Gritz et al., 1975; Specka et al., 2000).

Topological aspects of functional connectivity

In contrast to the healthy controls which had quite symmetrical topography of the most representative and stable functional connections, the patients exhibited quite different picture. The most stable topological combinations of functionally synchronized areas in methadone-treated patients were located in the frontal, central-temporal, and left parietal cortical poles (Fig. 3), probably reflecting those cortical circuits which are involved in the physical dependence. This finding is consistent with the work of Gritz et al. (1975).

Conclusions

The present preliminary study demonstrated that average parameters of temporal and metastable organization of the cortical dynamics (indexed by local and remote EEG functional connectivity) in opioid-dependent patients did not differ from normal values of healthy subjects after at least six-months (on average 11 months) of methadone treatment. To our knowledge, present study is the first where the capability of the methadone to restore normal temporal and metastable structure of brain activity was demonstrated explicitly. Additionally, in our previous study (Fingelkurts et al., 2007b) we have shown that methadone can restore also the temporal characteristics of brain oscillations. Altogether these findings support the view that methadone maintenance treatment may be indeed an effective management strategy for opioid dependence (Brands & Marsh, 1997).

However, there were still small but significant differences in the individual parameters of functional connectivity in methadone-treated patients when compared with healthy subjects. Considering that methadone does cause physical dependence, but has fewer problems with psychological dependence (Ferrari et al., 2004), we may speculate that probably the significant

differences found in the local and remote functional connectivity between methadone treated patients and healthy subjects in the present study, most likely indicate the neural correlates of physical opioid dependence. On the contrary the vast majority of significant differences found during acute opioid (Fingelkurts et al., 2006) and abstinent (Fingelkurts et al. 2007a) conditions between patients and healthy subjects reflect rather the neurobiological underpinnings of the biased cognitive (psychological) processes during addiction.

At the same time, preliminary data presented in the current paper do not allow making the firm conclusion on whether the reported results were determined by the methadone-maintenance treatment alone or together with the long (at least 6 months) withdrawal from heroin and buprenorphine consumption. Indeed, several studies demonstrated that long (at least 3 months) withdrawal without any specific treatment results in considerable or even complete normalization of EEG parameters (Bauer, 2001; Gekht et al., 2003) and cognitive functions (Selby & Azrin, 1998). However, neither temporal, nor functional EEG connectivity was the subject of those studies. Probably, the combination of methadone treatment together with withdrawal may play a role. Examination of a particular contribution of each of these factors remains for further investigation.

Another limitation of this study was the sample size. Since only six subjects participated, the present study should be considered as preliminary. However, the fact that all subjects have very similar methadone-induced changes in the EEG parameters stresses the validity of the presented results.

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