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Electroencephalographic and Immunological Findings in Patients with Schizophrenia Treated with Clozapine

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Academic Dissertation

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To my family
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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications, which will be referred to in the text by Roman numerals I to VI:


ABBREVIATIONS AND SOME DEFINITIONS

APA = American Psychiatric Association
BPRS = Brief Psychiatric Rating Scale
CLO = clozapine
CNS = central nervous system
D1-5 = dopamine receptors, types 1-5
EEG = electroencephalography
FC = fronto-central scalp area
FCleft = left fronto-central scalp area (EEG electrodes Fp1, F3, C3)
FCright = right fronto-central scalp area (EEG electrodes Fp2, F4, C4)
FCz = midline fronto-central scalp area (EEG electrodes FpZ, FZ, CZ)
FFT = Fast Fourier Transformation = an algorithm used in digital signal processing that break down complex signals into elementary components (www.pcmag.com/encyclopedia)
GABA = gamma-aminobutyric acid = an inhibitory neurotransmitter
IL = interleukin = family of proteins modulating immune response
MENTAL DISORDER = concept comprises a broad range of problems, generally characterized by some combination of abnormal thoughts, emotions, behaviour and relationships with others (www.WHO.int)
MENTAL ILLNESS = chronic mental malfunction, when people's capacities to respond to the world, to absorb and remember information, respond with appropriate emotions, and form coherent plans are impaired (www.answers.com / Philosophy Dictionary)
MO = peripheral blood monocytes
MOn = peripheral blood monocytes, not stimulated
MOs = peripheral blood monocytes, stimulated
NO = nitric oxide = signalling molecule and reactive oxygen species
PANSS = Positive and Negative Syndrome Scale
PANSSG = PANSS general psychopathology scale
PANSSN = PANSS negative scale
PANSSP = PANSS positive scale
PMA = phorbol myristate acetate = a substance which *in vitro* triggers cellular immune reactions in immune cells
QEEG = quantitative electroencephalography = quantitatively analysed digital EEG record
ROS = reactive oxygen species = molecules or ions that contribute to the microbicidal activity of phagocytes, regulation of signal transduction and gene expression, and the oxidative damage to nucleic acids; proteins; and lipids (http://ghr.nlm.nih.gov/ghr/glossary)
SCH = schizophrenia
SUMMARY

This work studies the effect of clozapine (CLO) on the electroencephalography (EEG) and reactive oxygen species (ROS) production by peripheral blood monocytes (MO) in patients with schizophrenia (SCH). The aim of the study was to investigate the mechanism of action of CLO, to clarify the effect of CLO on EEG absolute power spectrum and ROS production, and explore the relationship of these effects with clinical response. We also tried to clarify whether the EEG changes or ROS production would help to identify the patients who were most likely to respond to treatment with CLO.

Our findings suggest that the amount of slow background activity, particularly the absolute power of the theta frequency band, in the EEG is markedly increased by CLO treatment and this finding correlates positively with clinical improvement in patients with SCH. CLO affected the production of ROS by blood MO with reduction or minimal increase of the ROS production being associated with clinical improvement, whereas marked increase of the ROS production did not. Also a positive correlation between theta absolute power increase in the EEG and suppression of the production of ROS by blood MO was found. The correlations between different symptom clusters of SCH and the EEG rhythms were investigated; the absolute power of beta activity in the EEG seemed to correlate positively to overall psychopathology in patients with SCH showing inadequate response.

The results suggest that the EEG background activity and investigation of the production of ROS by MO seem to be an adjunctive method to objectively assess and possibly predict the therapeutic effect of CLO in patients with chronic SCH showing inadequate response to treatment with conventional antipsychotics.
1. INTRODUCTION

1.1. Schizophrenia

1.1.1. General description and etiology

SCH is discussed in most cases as if it were a single illness. The diagnostic entity includes, however, a group of heterogeneous disorders with different courses, etiologies and responses to treatment (Sadock & Sadock 2003), even though they present with somewhat similar symptoms. In many cases the illness impairs substantially a patient’s ability to form social relationships, to acquire skills needed for daily living and working, as well as to organise their time and activities. It may also reduce their drive and motivation, distort perception and cause disorganization of thinking and decline in cognitive function. The implications of the impact of SCH on the quality of life of the patients and their relatives are severe because of the relapsing course of the illness, emotional burden, social stigma and marked decline in daily functioning of the patients. There is also an increased mortality associated with SCH (Barbato 1998).

The etiology of SCH still remains obscure. Several hypotheses have been raised over the years. The significance of genetic factors has been shown in twin studies with probandwise concordance rates of 41-65% in monozygotic twins and heritability estimates of approximately 80-85%. (Cardno et al 1999, Cardno & Gottesman 2000). The research has shown that in addition to genetic factors the environmental factors play a substantial role (30-50%) in the etiology of SCH – problems with maternal bonding, early rearing in poverty, immigration status, stress, complications during pregnancy and during birth, and perinatal viral infections contribute to the increased risk of SCH (Sadock et al 2000). Tienari et al (2003) showed an increased prevalence of SCH in adopted-away off-springs of mothers having SCH or SCH spectrum disorder.

The neurodevelopmental hypothesis is supported by a growing body of data and according to this hypothesis the neuronal cell birth, differentiation, migration,
synaptogenesis, programmed cell death and neuronal circuitry formation have been altered leading to behavioural and cognitive dysfunction (Bunney & Bunney 1999, Eastwood et al 2003).

A “two hit” model has been proposed to explain the onset of SCH in early adulthood. The first hit consists of an early (antenatal) disruption of neural development and the second hit is an environmental stress during childhood/adolescence (McCarley et al 1999, Schiffman et al 2002). The hitting factors are considered to affect the brain’s development and maturation ultimately leading to the development of SCH.

There has been no single causative factor identified and the strongest evidence suggests SCH being an inherited disorder which is exacerbated by external stress. The broad dysfunction of the brain neural networks seems to be present in patients with SCH affecting to some degree the majority of their mental functions.

1.1.2. Prevalence of schizophrenia

SCH is a mental illness affecting approximately 1% of the World’s population (Schultz & Andreasen 1999, Norquist & Narrow 2000) although a wide range (0.3 - 17 per 1000) of prevalence has been reported in different studies (Torrey 1987) and (4-7 per 1000) according systematic review Saha et al (2005). Studies have suggested that SCH appears to be slightly more prevalent in Finland than in most other western developed countries (Torrey 1987, Lehtinen et al 1990, Hovatta et al 1997, Suvisaari 1999, Perälä et al 2007). In Finland the prevalence of 1.3% was shown in the Mini-Finland Health Survey (Lehtinen et al 1990). In the recent publication of Perälä et al. (2007) a prevalence of SCH of 0.87 % was shown but SCH, schizoaffective disorder and schizophreniform disorder showed
a combined prevalence of 1.26%. The yearly incidence was 0.1-0.4 per 1000 and the mean age of onset was 24 years (Sham et al 1994).

1.1.3. History, diagnosis and symptom clusters

Some forms of psychosis have been described since ancient times. Galen (131 - ca. 200 AD) used the term “insanity” which may have referred to SCH among other psychotic conditions. SCH as an illness was only clearly differentiated from other psychotic illnesses and conditions at the end of the nineteenth century by Emil Kraepelin. Kraepelin named the conditions characterised by early onset and the permanent deterioration of mental functioning as Dementia Praecox in 1893 (Harms 1971). He emphasised the gradual deterioration and decline of intellect, the weakening of volition and the emotional dullness caused by the illness. Kraepelin (1919) described the characteristic symptoms of SCH, not considering any single symptom, as pathognomonic for SCH. Kraepelin (1919) wrote: “Dementia praecox consists of a series of states, the common characteristic of which is a peculiar destruction of the internal connections of the psychic personality. The effects of this injury predominate in the emotional and volitional spheres of mental life.”

The diagnosis “schizophrenia” was proposed by Eugen Bleuler in 1911 and has been in use since. He wrote that the “splitting” of the different psychic functions is one of the most important characteristics of the illness. The impairment of the volition and emotions as well as the disorganisation of thinking was emphasized as the main characteristic of this illness by both - Kraepelin and Bleuler. Bleuler (1911) believed, however, that the disease includes probably several illnesses. Bleuler (1911) described the fundamental symptoms of SCH being disordered association, inadequate and flat affect, ambivalence, and autism. Fundamental symptoms of the illness remain observable also between relapses.
Schneider (1959) considered delusional perception, auditory hallucinations, passivity of thought and passivity experiences as being the First Rank Symptoms, which have also been used as fairly reliable criteria for diagnosis in clinical work. The persistent delusions and auditory hallucinations have been also considered as the most specific diagnostic criteria (Schneider 1959, Feighner 1972, Spitzer 1975, DSM-III-R 1987).

Kay et al (1987) clustered symptoms to positive, negative, and general and this symptom classification has become widely accepted in scientific research as well as in clinical work. The different symptom clustering of disorganisation, reality distortion, and psychomotor poverty proposed by Liddle (1987) has also been widely used in scientific research. Depending on which symptom cluster is dominating in the clinical picture, it can give some indication of potential differences in the course of the illness (Ho et al 2004), to some extent predict the response to treatment and make the prognosis. Patients with predominantly positive symptoms generally respond better to pharmacotherapy than patients with predominantly negative symptoms and marked cognitive deficit (Andreasen et al 1990). According to Liddle's classification the reality distortion and the disorganisation symptom clusters respond to conventional antipsychotics whilst psychomotor poverty cluster generally has a limited response.

Diagnosis of SCH as a single entity, even today, remains challenged. The diagnosis of SCH is descriptive and is based on the structurally described psychiatric symptoms forming a nosological entity. The first classification implementing clear-cut descriptive criteria for psychiatric diagnoses and introducing the concept of mental disorder was Diagnostic and Statistical Manual, Third Revision (DSM-III) published by APA in 1980. Schizophrenic disorder was used in DSM-III but in the description of the disorder the term illness was still used (DSM-III 1980). Descriptive criteria used in DSM-III and in the revised version DSM-III-R (1987) became widely used diagnostic tools in clinical practice and research work both within and outside of the USA.
Although the etiology of SCH is not yet known, the concept of schizophrenia as an illness is commonly used and is also used in this introduction along with the term disorder.

To date there are still no specific biological trait markers to verify the diagnosis of SCH. The diagnostic concepts of SCH presented by different sources differ to some extent even today and there is no total consensus regarding the core symptoms of the illness among psychiatrists.

1.1.4. Brain pathology in patients with schizophrenia

SCH has been considered a functional brain illness. Patients with SCH lack gross brain pathology but the hypothesis of minor morphological changes had been suggested by E. Kraepelin as early as 1905 (Rajarethinam et al 2005). The findings are localised predominantly in the fronto-temporal cortical areas, thalamus and hippocampal formation (Staal et al 1998 and 2001, Ettinger et al 2001, Gaser et al 2004, Sim et al 2006). The pattern of abnormalities is suggestive of a disturbance of connectivity within and between these regions, most likely originating during brain development (Harrison 1999). At the same time, however, other studies do not support the presence of morphological changes (Hazlett et al 1999, Bagary et al 2002).

The neural network modulation involves mutual links between different neuromodulatory systems making the neural network as a whole sensitive to the changes even in one modulatory system. The balance of dopaminergic, serotonergic, glutamatergic, and gamma-aminobutyric acid (GABA)-ergic neuromodulatory systems are most probably all affected in patients with SCH (Byne et al 1999, Javitt et al 2000, Javitt et al 2005). The most investigated neuromodulator in patients with SCH is indisputably dopamine. Dopamine has been shown to regulate local network activity in animal experiments (Honkanen
Both families of dopaminergic receptors (D1-like family (D1 and D5) and D2-like family (D2, D3, D4)) are considered to be affected in patients with SCH (Seeman et al 1989). The dopaminergic activity seems to be overactive subcortically (Breier et al 1997, Abi-Dargham et al 2000, Kienast & Heinz 2006) but at the same time there is growing evidence of insufficient dopaminergic activity and compensatory upregulation of cortical D1 receptors in the frontal cortical areas (Weinberger et al 1988, Abi-Dargham & More 2002, Hirvonen et al 2006). The increased density of D2 receptors in patients with SCH was shown by Sedvall & Farde (1995). The loss of D3 mRNA expression from other dopamine receptors in the parietal and motor cortices of postmortem brains of patients with SCH was shown by Schmauss (1993) and a six-fold increase in the density of D4 receptors in patients with SCH was found by Seeman et al (1993). The reasons and consequences of these findings still remain subject of scientific speculation. The receptors’ regulatory function in the central nervous system is also difficult to explore due to their plasticity and mobility in and out of synaptic space (Triller & Choquet 2005).

Changes in neuromodulation possibly lead to the impaired integration of brain functions which clinically manifests with disorganisation of thoughts, inadequate affect, cognitive defect, disturbed odd behaviour and poor social functioning in patients with SCH. As several mediatory systems have been affected in SCH it might speak in favour of antipsychotics, which affect simultaneously several neuromodulatory systems, in treatment of SCH.
1.1.5. Treatment of schizophrenia

Along with diagnostic questions the treatment of SCH remains a significant clinical challenge. Substantial progress in the treatment of SCH has been achieved during the last five decades but the illness still remains incurable. The treatment of the illness lasts for years and often requires a multidisciplinary approach. The course of the illness involves fluctuations in the intensity of the clinical symptoms. The patient's insight is crucially important to ensure the patient's adherence to treatment. Unfortunately there are still 30% of patients who, despite all treatment efforts and even with good adherence, respond poorly to treatment and experience relapse within a year of treatment (Steingard et al 1994). Although there is no cure for SCH available, the treatment of the illness markedly reduces the symptoms and improves the social functioning of patients (Breier et al 1987, Swartz et al 2007).

The antipsychotic medication affecting dopamine receptors in the brain has been in clinical use since the 1950-s. The results of treatment with drugs having antagonistic properties on brain dopamine receptors have been commonly used and the most effective form of treatment to date. According to research in the acute phase of illness antipsychotics effectively reduce positive symptoms in roughly 70 percent of patients and maintenance therapy reduces the risk of relapse (Dixon et al 1995).

About 70% of patients with schizophrenia achieved remission compared to ~25% patients receiving placebo treatment (Sadock & Sadock 2005). Some response to pharmacological treatment of new cases of SCH was reported being as high as 87% (Robinson et al 1999). The illness tends to relapse in 96% of cases after discontinuation of antipsychotic medication during 2 years according to Gitlin et al (2001). In other studies about 20 % of patients with SCH fail to respond to antipsychotic therapy (Kerwin & Bolonna 1995) and at the same time only 13.7% of subjects met full recovery criteria for 2 years or longer in the
study of Robinson et al (2004). These results indicate that about two thirds of the patients probably respond to the antipsychotic treatment partially.

The non-adherence with treatment can contribute to treatment resistance. The satisfactory compliance rate in new cases of SCH treated with conventional antipsychotics was only 54% (The Scottish Schizophrenia research group 1987), Gilmer et al (2004) showed full adherence in 41% of beneficiaries with SCH. In many cases, however, the reason for the different responses to treatment remains obscure and the response to treatment for each patient individually remains difficult to predict despite good adherence. Treatment with antipsychotics has often been criticised because of the neurological side effects and limited efficacy in some of the patients. Even though antipsychotic medication has a problematic side effect profile of neurological, metabolic, hormonal, and haematological side effects, the antipsychotics remain an important part of the treatment showing the most robust treatment effect on the intensity of symptoms and on the course of the illness. The search for more effective drugs with more benign side effect profiles remains a challenge for psychopharmacological research.

1.1.6. Treatment resistant schizophrenia

Treatment resistant SCH is defined as showing failed or very limited response to two antipsychotics, one of which is an atypical, administered in therapeutic dose and sufficient duration (Taylor et al 2005). From all patients suffering from SCH 10% to 30% show little or no response to antipsychotic medications (American Psychiatric Association 2004) and thus can be considered to be classified as treatment resistant. Treatment resistance may be a feature of the illness from its onset or it can develop in due course (Sheitman et al 1998). Beside ineffectiveness of medication the treatment resistance also might be related to comorbidity, substance misuse, poor adherence with treatment, and suboptimal dosing of medication (American Psychiatric Association 2004). The treatment
and management of treatment resistant SCH involves high doses of antipsychotics, combination therapy, CLO treatment, CLO augmentation and cognitive behavioural psychotherapy combined with psychoeducation. CLO, however, is still the treatment of choice for treatment resistant SCH (Taylor et al 2005).

1.2. Clozapine
1.2.1. Clozapine as an atypical antipsychotic

Along with conventional antipsychotics with high affinity for D2 blockade there are antipsychotics available with different pharmacological properties and side effect profiles. CLO was synthesized in Switzerland in 1958 and subsequently recognised as an antipsychotic in 1959 (Meyer & Simpson 1997, Hippius 1999). It was taken into clinical practice in 1972 but following eight reported deaths from agranulocytosis in Finland in 1975 (Idänpään-Heikkilä 1977) it was withdrawn from the market but was later reintroduced to clinical practice with safety guidelines. Currently it is generally used as a second line antipsychotic treatment for treatment resistant SCH.

CLO has been proven the most efficient antipsychotic to date, being effective in 30 - 50% of treatment resistant cases (Lieberman et al 1994, Wahlbeck et al 1999). The full mechanism of action of CLO remains obscure but alongside the effect on different dopamine receptors the widespread effects on multiple mediatory systems in the brain might have a therapeutic role. CLO also probably affects the psychoneuroimmunology and neurophysiology of the brain.

CLO has been considered as an atypical antipsychotic because it generally does not cause extrapyramidal side effects. The underlying reason is probably a limited binding to dopaminergic D2 receptors and it might also be related to CLO’s wide spectrum of action on many neuromediatory systems –

The effect of CLO on the dopaminergic system is also different from potent D2 blocking drugs as CLO has higher affinity to D4 receptors (Lahti et al 1993, Sanyal & Van Tol 1997, Seeman et al 1997). CLO’s clinically active metabolite N-desmethyl-clozapine has been investigated recently and there have been intriguing reports about broad activity of this compound on cholinergic M1 receptors and glutamate NMDA receptors (Sur et al 2003, Li et al 2005). N-desmethyl-clozapine possibly also acts as a partial agonist of dopaminergic D2 and D3 receptors similarly to the novel antipsychotic aripiprazole (Burstein et al 2005).

CLO still remains as a model for the newer atypical antipsychotics. Efficacy of CLO in treatment resistant patients has been shown in various studies. CLO has been shown to have an effect on suicidal behaviour (Meltzer and Okayli 1995) and almost all symptom clusters of SCH. Kane et al (1988) showed higher efficacy of CLO compared to conventional antipsychotics. Kuoppasalmi et al (1993) showed improvement in positive and negative symptoms after three to six months of CLO treatment in two third of patients with treatment resistant SCH. Improvement in positive symptoms by 1 month, in negative symptoms with improvement in social functioning by 3 months was shown in an open prospective trial by Jalenques et al (1992). The full clinical effect of CLO might in some cases appear only after 6 months of treatment (Meltzer et al 1990). According to Lieberman et al (1994) 50% of the patients with treatment resistant SCH and 76% of the treatment intolerant patients responded to 12 months trial of CLO treatment. Significant cognitive improvement, as measured by Wechsler
Adult Intelligence Scale-Revised (WAIS-R), was observed after a year of treatment with CLO in patients with treatment resistant SCH (Fujii et al 1997).

1.2.2. Side effects of clozapine

CLO is considered to be the first atypical antipsychotic as it does not generally cause neurological extrapyramidal side effects. At the same time the complication of metabolic side effects, epileptic seizures, leucocytopenia and potentially fatal agranulocytosis are related to CLO treatment.

Weight gain has been shown to be more prevalent with CLO treatment (Bustillo et al 1996). Increased risk of metabolic syndrome with a prevalence of 54% compared to 21% in the general population in the US has also been demonstrated (Lamberti et al 2006). Henderson et al (2000) showed that the patients treated with CLO experience significant weight gain, lipid abnormalities and appeared to have an increased risk of developing diabetes.

Risk of epileptic fits was 1 - 4.4% and is dose-related (Devinsky et al 1991). However the incidence of epileptic fits was reported being as high as 9.4% by Liukkonen et al (1992) and 20% by Welch et al (1994).

The risk of agranulocytosis and the substantial risk of death due to agranulocytosis only became evident in 1975 (Idänpää-Heikkila 1977). CLO has an inhibitory effect on the production of the blood granulocytes (Atkin et al 1996). Haematological problems related to CLO treatment have been reported in 3,6% of patients treated with CLO (Wahlbeck et al 1999) and the cumulative incidence of agranulocytosis after one year of CLO treatment was 0,8% (Alvir et al 1993). The reasons behind the development of agranulocytosis are not completely elucidated. Interesting results have been published recently by Bergemann et al (2007) who showed clozapine concentrations in the leukocytes of the patients who developed leukocytopenia eight times higher than those who
did not develop leukocytopenia. The elevated proapoptotic gene expression along with the ROS production in the neutrophils of the patients with CLO-induced agranulocytosis is reported by Fehsel et al (2005). The direct toxicity of CLO is not certain at therapeutic concentrations but CLO undergoes bioactivation to a toxic, chemically reactive nitrenium ion (Maggs et al 1995, Pirmohamed et al 1995) and Williams et al (2000) showed that the neutrophil apoptosis was induced at therapeutic concentrations of CLO when it was bioactivated to a nitrenium ion.

The haematological side effects remain the most concerning factors limiting the use of CLO in clinical practice. There are strict limitations and regulations applied to the use of CLO in clinical practice. In most countries a system for obligatory white cell count monitoring is established for the haematological risk management.

Emergence of metabolic side effects seems to coincide with achieving the desired clinical effects. Meltzer et al (2003) reported the increase in weight predicted improvement in psychopathology in patients treated with CLO.

1.3. Psychoneuroimmunology

1.3.1. General description and association with schizophrenia

Psychoneuroimmunology deals with two basic processes: influence of mental activity on regulation of immunological processes and influence of autoimmunological reactions on neural activity and brain function (Solomon 1987, Ader 2003). The CNS has influence over immunological function as well as regulation of hormonal production. Direct autonomic neural regulation has an effect on the immunological reactivity. There is a predominate sympathetic (mediated by catecholamines) input to all components of the immune system – e.g. thymus gland, spleen, lymph nodes, and bone marrow. Activation of the sympathetic
nervous system primarily inhibits the activity of cells associated with the innate immune system, while it either enhances or inhibits the activity of cells associated with the adaptive immune system. (Nance and Sanders 2007).

There is evidence supporting the hypothesis of association between inflammatory process and SCH, at least in some patients (Leonard 2005). Leonard (2005) also emphasised that the genetic link (chromosome locus 6p22) between human lymphocyte antigen system and SCH as well as the epidemiologic finding of negative correlation between rheumatoid arthritis and SCH also indicates the possible involvement of immune component in SCH.

Modern immunological methods have shown the innate as well as adaptive immune system to be involved in immune dysfunction in SCH. The increased number of MO/macrophages and activation of interleukine (IL)-6 indicate the activation of the innate system (Nikkilä et al 1999, Muller et al 1999). The elevated IL-6 serum levels have been found associated with duration of illness (Ganguli et al 1994) and the resistance to drug treatment (Lin et al 1998) in patients with SCH. There is evidence that the cellular arm (T-helper-1) of the adaptive immune system is reduced while the humoral arm (T-helper-2) is increased (Leonard 2005). The serum T-helper-2 shift, indicating relative activation of humoral type immune activity seems also to be specific for SCH (Muller et al 1999, Chiang 2005). According to existing data the effective treatment with antipsychotic drugs seems to restore the imbalance between these systems (Leonard 2005).

The controversial results of different studies investigating the contribution of cytokines (IL-2) in SCH were analysed by Hinze-Selch & Pollmacher (2001). They concluded that despite different methodologies and study designs the medication and cigarette smoking are likely to play a role in the immune system changes in patients with SCH.
Despite the controversial research results and complicated interactions between immunological mechanisms there is currently little doubt about the involvement of immunological mechanisms in SCH.

1.3.2. Free radicals and reactive oxygen species

Free radicals are highly reactive chemical species or molecules (Cadet 1988). By definition a free radical is any atom or molecule possessing a free unpaired electron in the outermost shell and is capable of independent existence (Karlsson 1997). This unpaired electron makes the free radical unstable and highly reactive. Free radical is “seeking” another balanced molecule from which to steal electrons. 90% of free radicals are produced in the mitochondria (Sidiropoulos 2005).

Free radicals involving oxygen can be called as reactive oxygen species (ROS). Most free radicals are ROS. The ROS family includes hydrogen peroxide (H₂O₂), superoxide (O₂⁻), hydroxyl (OH), peroxyl (ROO), alkoxy (RO), nitric oxide (NO), and peroxynitrite (ONOO). Limited amount of ROS are generated by normal cellular functions like arachidonic acid metabolism, mitochondrial respiratory chain, phagocytosis, ovulation and fertilisation. During pathological conditions the production of ROS multiplies several times and in excess they can damage lipids, proteins and DNA (Young et al 2007), increase lipid peroxidation (Phillips et al 1993) and alter anti-oxidant enzymes (Abdalla et al 1986). The toxicity and reactivity varies between different ROS. ROS may attack and damage the cell membrane or other structures that compose the cell by stealing electrons from the lipid membranes of a cell, which is known as lipid peroxidation (Helwig 2000). Additionally to cell membranes ROS can also damage or fragment DNA and modify proteins. These effects may ultimately lead to cell death.
ROS are mainly synthesized by immune cells and in the CNS by brain macrophages – microglia. Release of ROS has also been reported during the recovery phases from many pathological conditions in the brain (Fisher et al 1988, Halliwell et al 1989, Fischer et al 2001, Halliwell 2006). It has been shown that NO is generated also by glial cells (Halliwell et al 1985). It has also been speculated that particularly NO is implicated in some neurodegenerative illnesses in CNS.

1.3.3. Reactive oxygen species and schizophrenia

There is some evidence indicating the role of ROS in the pathogenesis of SCH (Reddy & Yao 1996, Mahadik et al 2001). Sirota et al 2003 reported a positive correlation of ROS (superoxide) production by neutrophils with negative symptoms of SCH. Increased serum levels of NO were recently reported in patients with chronic SCH by Yilmaz et al (2007).

The catecholamines (adrenaline, noradrenaline, dopamine) might be related to ROS generation in CNS, they can spontaneously break down or be metabolised by monoamine oxidase to ROS (Singh et al 2004). The increase of dopaminergic activity in SCH might lead to excessive breakdown of dopamine forming ROS and toxic quinolines. On the other hand dopamine has powerful antioxidant properties by several separate mechanisms – activation of the synthesis of antioxidative proteins, direct ROS scavenging and possibly acting via dismuting complexes (Smythies 2000). Smythies (1999) also showed that activation of D2 receptors induces the synthesis of antioxidant enzymes. He also proposed that the function of neuroprotective catecholamine-iron complexes or neurotoxic catecholamineo-quinoline complexes may be defective in SCH. Elevated sodium dismuthase activity has also been reported in patients with SCH, which is possibly an adaptive response to ROS overload as sodium dismuthase is a ROS scavenger (Abdalla et al 1986). Increased serum levels of sodium
dismuthase and thiobarbituric acid reactive substances (a measure of lipid peroxidation) have also been reported in patients with SCH (Gama et al 2006).

Cigarette smoking is common among patients with SCH and it has been shown to aggravate the ROS production (Gustafsson et al 2000). On the other hand Zhang et al (2007) reported decreased oxidative stress and lipid peroxidation in patients with SCH who smoked tobacco.

It seems that there is an increase in the level of free radical activity as well as antioxidants in the serum and possibly CNS of patients with SCH.

1.3.4. Reactive oxygen species and antipsychotic medication

There is contradicting data about the effect of antipsychotics on ROS production. The conventional antipsychotics have been shown in in vitro studies to produce an eight-fold increase in ROS production, whilst CLO and sulpiride did not induce a significant increase (Sagara 1998). Haloperidol increased production of ROS by mitochondria and antioxidants reduced cell death induced by haloperidol (Post et al 1998, Sagara 1998). In the same time serum levels of the thiobarbituric acid reactive substances (index of lipid peroxidation and oxidative stress) were significantly higher in CLO treated SCH patients than haloperidol treated SCH patients (Gama et al 2006). Bastianetto et al (2006) reported that CLO showed a neuroprotective effect but through a ROS independent and caspase dependent mechanism in hippocampal cells.

Regarding other atypical antipsychotics the recent results of Kato et al (2007) indicate that risperidone significantly inhibited the production of nitric oxide and proinflammatory cytokines by activated microglia. Hou et al (2006) reported that olanzapine but not CLO and haloperidol caused inhibition of NO production by microglial cells.
It seems to be the case that all antipsychotics, atypicals and CLO affect immunological mechanisms and apoptotic systems in the neurons as well as peripheral blood cells. It is, however, unclear to what extent this influence is related directly to antipsychotic effect and what relates to the neuropathology of SCH.

1.4. Electroencephalography
1.4.1. General description

Electroencephalography (EEG) is a non-invasive neurophysiological investigation method. EEG is a registration of brain electrical activity recorded from the surface of the scalp. The electrodes are attached to the surface of the scalp to pick up the electrical activity which is recorded by the EEG machine. Electrode placement is determined by measuring the head and marking the scalp. The 10-20 International System of Electrode Placement (Jasper 1958) (Appendix 2) is used by the great majority of EEG labs throughout the world (Hughes 1994). The numbers 10 and 20 refer to percentages of the distances between electrodes. There are 19 electrodes attached to the scalp and two to the earlobes as reference electrodes. The electrodes are called prefrontal, frontal, central, temporal, parietal and occipital electrodes and they record the electrical activity from the same cortical regions respectively.

Voltage differences between different parts of the scalp are measured in the EEG but not electrical currents (Fish 1999). The frequency of the spontaneous electrical activity has a range from less than 1 Hz up to 80Hz. The main frequencies investigated are in the range of 1 – 20 (25) Hz and are arbitrarily divided in to four ranges: Delta (1 – 3.5 Hz), Theta (4 – 7,5 Hz), Alpha (8 – 13 Hz) and Beta (13 – 25 Hz). The frequency band 25 - 35 Hz has been called Beta II and the frequency band above 35 Hz the Gamma frequency (Boutros & Braff 1999). The change in electrical activity can be noticed with a good temporal
resolution (1ms) and moderate spatial resolution (10-15 mm) (Volkow et al. 1996).

The EEG consists of the inhibitory and excitatory postsynaptic potentials generated mainly in the cortex of the brain (Fish 1999). The EEG is a result of summation of the electrical activity of the clusters of brain cells. The weak electromagnetic fields caused by depolarisation of every single brain cell will be summarised due to simultaneous rhythmic firing of the cells. The electrical impulses produced by the brain have a voltage of only tens of microvolts and thus the signal has to be amplified for the EEG registration. The brain electrical activity has to be filtered from various artefacts such as electrical activity caused by muscles, the heart or from possible external sources (Hughes 1999).

The brain has a complex cortical surface. The EEG registers the electrical activity from the closest cortical area and predominantly from the gyri which generate a radial electromagnetic field (Barkley & Baumgartner 2003). The pyramidal cells in the IV cortical layer are the main source of EEG activity. The rhythmic activity probably originates from the interaction of the brain cortex and thalamus (Fish 1999). In addition to neurons there are also glial cells involved in brain electrical activity, amplifying the electrical fields and affecting the spatial distribution of the fields (Boutros & Braff 1999).

The EEG has been used from the time of its discovery to diagnose epilepsy and to identify focal as well as generalised organic brain pathology (Hughes & John 1999, Smith 2005). The EEG can be analysed visually by an experienced neurophysiologist or it can be digitalised and quantified by computer which enables more detailed analysis of the EEG. The development of digital technology and more sophisticated mathematical analysis methods of EEG, such as quantitative EEG (QEEG), has opened new possibilities for the use of the EEG in diagnostic work (Hughes & John 1999).
1.4.2. Quantitative electroencephalography

QEEG is based on the quantitative analysis of the EEG signal. The “background” EEG signal from 21 attached electrodes at a standardised eyes-closed resting position is recorded, digitalized and stored. From the stored data a sample of 1 to 3 minutes of artefact-free EEG is visually edited and analysed. The powers of frequency bands of delta, theta, alpha, and beta rhythms are calculated using the Fast Fourier Transformation (FFT) mathematical algorithm. The averaged power spectrum values of the frequency bands are produced for each electrode across the entire sample, known as the power spectrum. Results can be represented as absolute power (total µV^2) or relative power (percentage of total power), there is also used coherence (phase synchronization of two channels), or symmetry (the ratio of power between a symmetrical pair of electrodes) (Hughes & John 1999).

The analysis of the QEEG power spectrum gives a detailed picture of the brain background electrical activity. According to Hughes and John (1999) studies have shown QEEG to be both a specific and sensitive method for investigating psychiatric disorders even though the method does not have currently clinical applications. On the other hand Nuwer (1997) expressed his concerns regarding overoptimistic application of QEEG in clinical practice but still acknowledged the progress which has been made in the scientific understanding of cerebral dysfunction in many disorders including SCH. QEEG is currently not approved for diagnostic purposes of psychiatric disorders (Coburn et al 2006), but could have corroborative value in making clinical pharmaco-therapeutical decisions.

QEEG could be useful in clinical practice for assessment of the efficiency of antipsychotic medication. Change in the absolute power spectrum of the theta frequency band in the QEEG has been shown to correlate with the antipsychotic effect of medication (Chobor et Volavka 1992, Omori et al 1995, Kikuchi et al 2005).
1.4.3. EEG background activity

Spontaneous rhythmic electrical activity is observed in the brain which is dependent on mental activity, emotional state, level of arousal and age. It is also influenced by psychotropic substances. The EEG varies substantially between different individuals but it remains, however, a relatively stable characteristic for one individual (Salinsky et al 1991, Williams et al 2005).

EEG background electrical brain activity is regulated by anatomically complex homeostatic systems. Cortical processes are modulated by the brainstem and thalamus using all the major neurotransmitters (McCormic1992, Lopez da Silva 1996). Different rhythms are believed to reflect different neurophysiological states. The cortical activity also most likely reciprocally influences subcortical structures to make possible fast switching from one functional state of the brain to another necessary for adequate responding to external stimuli.

The pacemaker function probably originates mainly from the brainstem and the rhythm is further modulated in the thalamus. The beta rhythm (13-20 Hz) is believed to reflect thalamocortical and corticocortical interneural transactions related to specific information processing. The alpha rhythm (8-13 Hz) is the dominating frequency in the EEG of an alert adult person at rest. Modulating neurons throughout the thalamus normally oscillate synchronously in the alpha rhythm which is globally distributed across the cortex. Gamma-aminobutyric acid (GABA) release by the nucleus reticularis diminishes sensory throughput by thalamic neurons to the cortex which is observed as a slowing in the dominant alpha rhythm into the theta range (4-7,5 Hz). Delta activity (1-3,5 Hz) probably originates from oscillator neurons in deep cortical layers and in the thalamus. These neurons are normally inhibited by the ascending reticular activating system in the midbrain (Hughes & John 1999). Slower rhythms are related mainly to deep sleep stages, states of unconsciousness, diffuse brain pathology as well as treatment with antipsychotics. Faster rhythms are related to anxiety, task solving and treatment with anxiolytics.
Changes in background activity related to mental disorders are generally subtle and often require quantitative analysis instead of visual impressionistic evaluation.

1.4.4. The EEG and Schizophrenia

The first recorded EEG of a patient with psychotic disorder was probably in Cambridge in 1936 (Lemere 1936). It was followed by reports of Berger (1937) who invented the EEG in the first place. Davis (1940, 1942) referred to “disorganisation” and “choppy activity” (probably consisting of a low voltage beta activity) in the EEG of psychotic patients. She also classified patients with SCH into 3 groups: Group I - Essentially normal. Group II: Dysrhythmic type, which is indistinguishable from EEG’s of individuals known to have convulsive disorders. Group III: "Choppy" type, which suggests the possibility of a pathological condition in the brain (Davis 1940).

Early reports of Jasper (1939) and later Goldstein (1965) reported low amplitude and prominent slow wave activity in EEG records of patients with SCH. Numerous qualitative studies have indicated abnormal EEG findings in range 20% to 60% of schizophrenic patients (Ellingson 1954, Small 1984, 1993, Sponheim 1994). According Hughes and John (1999) 68% of psychiatric patients’ EEGs, provide evidence of a dysfunction of the brain. In the same time Centorrino et al (2002) indicated the presence of EEG abnormalities in less than 20% of psychiatric inpatients.

The quantification of the EEG and the power spectral analysis enabled more detailed analysis of the EEG. Itil et al (1972) reported increased beta, theta and delta activity in patients with SCH. Whether EEG findings are state or trait related features in patients with SCH has been a matter of dispute for decades.
(Sengoku & Takagi 1998). Stassen et al (1999) found that the EEG abnormalities associated with SCH manifested differently in co-twins concordant for SCH, and suggested that it reflects the non-genetic, pathological developments of genetically identical brains. Winterer et al (2001) supported this with his findings and proposed that power spectrum EEG abnormalities may be state-dependent in patents with SCH. Equally there are features in the EEG that might reflect genetic vulnerability and should be considered as a trait related characteristics. These parameters are coherence (Winterer et al 2001), reduced amplitudes of auditory evoked potentials (Weisbrod et al 1999, Ahveninen et al 2006) and probably reduced pre-pulse inhibition of the startle response (Hamm et al 2001). The current data suggests that the state dependant and the trait related changes probably both exist in the EEG of patients with SCH.

Different symptom clusters have correlated with EEG changes. Negative symptoms have correlated with an increase of delta (Guenther et al 1988, Gattaz et al 1992) and beta band (Williamson et al 1989) activities. Karson et al (1988) and Sponheim et al (2000) showed that increased low-frequency power and diminished alpha-band power was associated with negative symptoms, enlarged ventricles and cortical atrophy. Positive symptoms were correlated to theta and delta activities in a magneto-encephalographic study (Fehr et al 2001). Harris et al. (1999, 2001) reported correlations between QEEG frequency band powers, Liddle's three factors (Liddle 1987) of psychomotor poverty, disorganisation, and reality distortion and the negative and positive subscales of the Positive and Negative Syndrome Rating Scale (PANSS) (Kay et al 1987). Liddle's factors showed positive correlations with delta, alpha, and beta bands. The PANSS negative subscale correlated positively with delta power.

Topographically the most robust EEG findings have been reported predominantly over the anterior, temporal and central regions in patients with SCH. The correlations of symptom clusters with dysfunction of particular cortical areas is mainly localised to the fronto-temporal area (Itil et al 1972, Barta et al 1990,
Kawasaki et al 1996). Treatment resistant patients with SCH exhibited greater overall absolute theta power, slower mean alpha frequency and elevated absolute delta and total power in the anterior regions (Knott et al 2001). Positive and negative SCH were found to differ only in the delta and theta bands over frontal regions (Begic et al 2000).

Psychiatric symptoms are most likely a consequence of dysfunction of multiple cortical areas and sub-cortical brain structures in patients with SCH.

1.4.5. The EEG and antipsychotics

The effects of medication on the EEG background activity were reported soon after antipsychotics were discovered (Jorgensen & Wulff 1958, Itil 1968, 1972). Antipsychotics have been shown to increase alpha power (Galderisi 1994, Saletu 1994, Schellenberg 1994) and reduce beta power in both short and long term administration (Herrmann 1986, Niedermeyer 1987, Hughes & John 1999) reversing some findings reported in patients with schizophrenia. Abnormal EEG changes have been described in hospital patients (with various diagnoses) on antipsychotic treatment. Abnormalities occurred in 19.1% of patients treated and in 13.3% of patients not treated with antipsychotics. Abnormality rates among patients taking conventional antipsychotics ranged from 36.4% with trifluoperazine to 7.3% with haloperidol, with intermediate rates in other antipsychotics of high or low potency (13%–14% with chlorpromazine, perphenazine, or thioridazine) and in subjects not treated with antipsychotics (13.3%) (Centorrino et al 2002).

Max Fink (2002) emphasised that the term "abnormality" comes from neurological literature that uses visual impressionistic methods to assess EEG records. But psychoactive drugs induce only subtle changes that are not always detected by visual analysis. The quantitative digital computer processing is an adequate method for detecting the effect of drugs on EEG (Fink 1985).
Therefore he considered the description of the EEG changes associated with psychoactive drugs as “abnormal” or “normal” as misleading.

The EEG changes caused by antipsychotics appear to relate to their clinical effect. Antipsychotic medication has been shown to attenuate beta frequency power particularly in patients responding to medication (Itil et al 1972, Guenther et al 1988). Change in the absolute power of the theta frequency band in the QEEG has been shown to correlate with the antipsychotic effect of medication (Chobor et Volavka 1992, Kikuchi et al 2005). Antipsychotics having different properties also seem to have different effects on the EEG. The modern atypical agents, increasingly used in clinical practice, have variable effect on the EEG. The risk of EEG abnormality was highest with CLO (47.1%), followed by olanzapine (38.5%) and risperidone (28.0%), with a few quetiapine treated patients having no abnormalities (Centorrino et al 2002).

The predictive value of the EEG on clinical effect has been the subject of numerous studies.

Pretreatment beta power and asymmetries in delta and theta were associated with overall clinical improvement (Czobor & Volavka 1993). Galderisi et al 1994 showed that the patients showing the same response in QEEG as healthy subjects (i.e. increased theta and alpha1 activity) six hours after being given a test dose of haloperidol or clopenthixol had more favourable clinical response to treatment.

Pharmaco-EEG has the potential for clinical applications. Several QEEG studies have shown consistent findings on early predictors of treatment response to first generation antipsychotics but the findings have so far not had clinical impact (Mucci et al 2006).

The pathological EEG findings in patients with SCH are affected by antipsychotic treatment. These findings may indicate further deterioration of brain function or reflect reparative or compensatory mechanisms and this remains a subject for future research.
It seems evident that the effect of CLO on the EEG is greater than that of other antipsychotics (Small et al 1987, Centorrino 2002). CLO induces an increase of slow frequencies in the background EEG activity in patients suffering from SCH (Tiihonen et al 1991, Guenther et al 1993, Risby 1993, Knott et al 2001) as well as in healthy volunteers. This effect was seen even after single dose of CLO administration (Saletu et al 1987, Galderisi et al 1996), and is greater than that seen in the newer atypical antipsychotics (Schuld et al 2000, Centorrino et al 2002). The degree of pathological findings in the QEEG has been suggested to correlate positively with the degree of clinical response to CLO treatment (Risby et al 1995), and with CLO plasma levels (Haring et al 1994, Freudenreich et al 1997). Stevens (1995) rise a hypothesis of the important role of subcortical structures in development of psychosis and presented an argument for evaluating the EEG slow waves caused by CLO as evidence of its therapeutic activity. The EEG changes after CLO, especially when instrumentally quantified, demonstrated the predictive value of the EEG (Roubicek & Major 1977). CLO is also known to give rise to epileptiform disturbances as well as disturbances in the background activity on EEG (Tiihonen et al 1991, Guenther et al 1993, Risby et al 1993, Treves & Neufeld 1996, Alper et al 2007). The CLO effect on brain neurophysiology is marked probably due to the affinity of the drug to multiple mediatory systems. The high antipsychotic efficacy seems to be related to widespread effect of CLO on various neural networks.
2. AIMS OF THE STUDY

In this work, the effect of CLO on the EEG and ROS production in patients with SCH was studied. The aim of the study was to clarify the effect of CLO on EEG power spectrum and ROS production by MO and explore the relationship of these effects with clinical response. We also tried to clarify whether the EEG changes or ROS production by MO would help to identify the patients who were most likely to respond to treatment with CLO.

The specific aims of the studies were:

I – II To investigate prospectively the association between effect of CLO on ROS production by MO and clinical outcome in patients responding inadequately to conventional antipsychotics and the temporal correlation of these changes.

III To study the QEEG changes characteristic to CLO treated patients compared to patients treated with other antipsychotics and healthy volunteers. QEEG absolute power spectrums between groups of patients receiving various antipsychotics with patients receiving CLO medication and healthy controls were compared.

IV To study changes in ROS production by MO and the association with the QEEG absolute power spectrum in patients responding inadequately to conventional antipsychotics and being treated with CLO.

V To investigate prospectively the association between clinical outcome and QEEG power changes in patients responding inadequately to conventional antipsychotics and being treated with CLO.

VI To study the association between QEEG main frequency absolute powers and different symptom clusters of psychiatric symptoms in patients showing minimal or no clinical improvement to treatment with conventional antipsychotics.
3. SUBJECTS AND METHODS

3.1. Patients and study design

3.1.1 Patients

The main characteristics of subjects and patients being investigated in studies I – VI have been presented in Appendix I.

3.1.1.1 Patients in the studies I, II, and IV

Eight inpatients from Tammiharju Mental Hospital (mean age 31.4 years) with chronic SCH (according to DSM-III-R) responding inadequately to treatment with conventional antipsychotics were studied. Patients did not have organic brain damage, neurological disorders, allergic states, acute infections, hyperthermia (>37°C), history of alcohol or drug-abuse, previous history of CLO treatment or any medication beyond the patients continuous psychiatric medication used within the last 2 weeks (3 months for steroids and antibiotics). The patients had previously received conventional antipsychotics (mean dose 386 mg chlorpromazine equivalents. Before baseline testing and QEEG recording there was at least a 36-hour wash-out period. Treatment with CLO started with a dose of 50 mg/day. The dose was thereafter increased stepwise by 50mg every third day. The final treatment dose was chosen according to the clinical need and the decision of the psychiatrist responsible for the treatment of the patients. Benzodiazepines and chloral hydrate in minimal effective doses as sleeping agents were allowed as if needed medication.

3.1.1.2 Patients in study III

A group of 21 patients with chronic SCH receiving various antipsychotics (non-CLO group), a group of 21 patients with chronic SCH receiving CLO treatment (CLO-treated group) and a group of 29 healthy volunteers were investigated.
The mean age in the CLO-treated group was 37.6 years, the average duration of illness was 15.7 years, duration of treatment with antipsychotics 15.5 years, and the mean dosage of CLO was 514 mg/day.

The mean age in the non-CLO group was 39 years, average duration of illness was 13.7 years, mean duration of treatment with antipsychotics was 13.2 years. Patients were medicated with one to three antipsychotic drugs simultaneously, the average dose being 405 mg chlorpromazine equivalents. Patients with any organic brain lesion, history of heavy alcohol or drug abuse, concomitant neurologic illness, organic psychosis or other psychotic disorders were excluded.

The control group consisted of 29 subjects (mean age 35 years), none of whom had psychotropic medication. They were healthy volunteers from the hospital staff without any neurological or psychiatric disease.

3.1.1.3 Patients in studies V and VI

The sample, who consisted of sixteen inpatients (mean age 32.6 years) suffering from SCH considered as having insufficient clinical response to conventional antipsychotic treatment and evidencing a relapse of the illness with PANSS mean total score 103 (range: 67–137), were investigated prospectively. The mean duration of illness was 10.4 years and they were previously treated with conventional antipsychotics corresponding to 386 mg chlorpromazine equivalents for 9.4 years on average. None of them had received CLO prior to the study. Before baseline investigation there was at least a 24 (up to 72)-hour washout period from previous conventional antipsychotic treatment. Treatment with CLO was started after the baseline visit and the same protocol was followed as in the studies I, II and IV with a dose of 50 mg/day. During the study the use of other psychoactive drugs, except benzodiazepines and chloral hydrate as hypnotics, was not allowed. The benzodiazepines were not allowed in the evenings preceding the QEEG registrations. Patients did not have neurological disorders or a history of alcohol or drug abuse.
3.1.2 Study Design

3.1.2.1 Design of studies I, II, and IV

The studies were designed as a 10 weeks prospective setting. The correlation between the increase of theta power in the EEG and ROS production by MO was prospectively investigated in eight patients with SCH and receiving CLO. Inpatients from Tammiharju hospital who fulfilled the inclusion criteria and gave informed consent were investigated. There was no control group. Patients were started on CLO treatment and were investigated on three occasions; before treatment with CLO, three and ten weeks after the initiation of the CLO. Assessments consisted of QEEG registration and Positive and Negative Syndrome Rating Scale (PANSS) (Kay 1987) conducted by a psychiatrist. In the EEG the midline frontal electrode (Fz) was chosen to investigate on the basis of our earlier research. Blood sampling for the investigation of ROS production and CLO blood levels were done on the same day. The results of the assessments were calculated and analysed only after completion of all assessments and data collection.

3.1.2.2 Design of study III

This was a cross sectional study where two groups of patients were compared with age matched healthy controls. The patients receiving CLO in various doses were included in one group, the patients with other antipsychotic medication and not having CLO, were included in another group. The QEEG investigations and power spectrum analysis results were compared between all three groups.
3.1.2.3 Design of studies V and VI

The study V was done in an open label prospective setting. Sixteen patients who were started on CLO treatment during the study recruitment period in Tammiharju Hospital, fulfilled the inclusion criteria and had given consent were investigated. There was no control group. The patients were investigated on five occasions; before treatment with CLO, after one, three, ten and sixteen weeks after the initiation of the CLO. Assessments consisting of QEEG registration and PANSS were conducted by a psychiatrist. Blood samples were drawn in the morning (before the first CLO dose of the day) for the investigation of CLO plasma levels. The results of EEG power spectrum analysis were correlated to PANSS results. The results of the assessments were calculated and analysed only after completion of all assessments and data collection. PANSS ratings of patients were performed blind to the QEEG results.

The same patients were investigated in the study VI using data from visit 1 before treatment with CLO was started. The results of EEG power spectrum analysis were correlated to PANSS subscale results and also to Liddle's three symptom clusters calculated from PANSS items. The antipsychotics were suspended for 12-36 hours before clinical assessment and QEEG.

3.2 Blood sampling, isolation of mononuclear leukocytes and reactive oxygen species assessments
3.2.1 Studies I, II and IV

Blood samples were taken between 07.45-08.00 a.m. during every visit (smokers’ samples were taken immediately after the first cigarette of the day) before the first CLO dose of the day. Total leukocyte counting was performed with a Counter Coulter (Counter Electronics, UK) from anticoagulated blood within 1 hour of blood sampling. At the same time blood smears were made for
differential count. Samples were calculated blindly and thereafter the results were analysed.

Polymorphonuclear leukocytes and mononuclear leukocytes were isolated using 10 ml of heparinised venous blood, layered on 7 ml Ficoll-Paque (Pharmacia, Sweden) within one hour of blood sampling and centrifuged at 450 g for 20 min at 20°C. The band in the interface between the plasma and the Ficoll layer, containing lymphocytes and MO, was aspirated and washed once in phosphate buffered saline. The leukocyte count was determined with the Counter Coulter, and the cell suspension was diluted to 5 x 10⁶ mononuclear cells/ml. Cytocentrifuge preparations of the cell suspension were prepared immediately, dried in room air and stained with May-Grünwald-Giemsa for determination of the MO proportion. The mononuclear cell suspension (50 μl) was added to a solution containing 5.6 mM luminol (Bio-Orbit, Finland). One half of the samples were stimulated with 0.1 μg/ml phorbol myristate acetate. The light emission caused by the production of ROS, mainly hypochlorite (DeChatelet 1982), was recorded for 30 min at 2-min intervals with a Bio-Orbit 1251 luminometer (Bio-Orbit, Finland). All measurements were performed in duplicate. The results are expressed as the areas under the light emission curves per 100,000 cells. The MO results were corrected for the actual MO count on the basis of the differential count obtained from the cytocentrifuge preparations. At baseline, before treatment with CLO, ROS production was also studied by adding CLO dissolved in water 5 mg/l after incubation at 37°C for 1 hour.

3.3 Quantitative EEG Recordings
3.3.1 Quantitative EEG recording and data selection in studies III, IV, V, and VI

QEEG recordings were performed in the neurophysiology laboratory in Tammiharju Hospital. Cadwell Spectrum P32 equipment was used. EEGs of the patients were recorded digitally as part of clinical work. Healthy controls were
investigated in the same laboratory using similar settings. QEEG recordings were performed between 9 a.m. and 1 p.m. During recording the subjects were awake with their eyes closed. The alpha frequency amplitude in the posterior parts of the brain was visually monitored and regular verbal contact with the subject was taken to control the vigilance. The EEGs were analysed afterwards. The standard 10–20 electrode placement system with 21 electrodes on the scalp and one on both earlobes was used in recording. The sampling rate was 200 Hz, with a low-pass limit of 70 Hz, and a time constant of 0.3 second. The digital recordings 10-20 minutes in duration were saved on optical disks.

The topographical quantitative calculations were made using the Cadwell Spectrum P32 Neurometrics program (Cadwell Laboratories, Kennewick, WA), with the ear electrodes as a reference. The digital EEG recordings were screened visually and 48 2.5-second artefact-free epochs were selected from the typical background for subsequent analysis. As a result of the Fast Fourier Transformation, the averaged power spectral values of the delta (1.5-3.0 Hz), theta (3.0-7.5 Hz), alpha (7.5-12.5 Hz), and beta (12.5-20.0 Hz) bands were produced for each of the 21 scalp electrodes separately.

3.3.2 Quantitative EEG calculations
3.3.2.1. Quantitative EEG calculations in study III

The absolute power values of all four main frequency bands at each electrode location were compared respectively and the correlations statistically tested between the CLO group, the non-CLO group, and the healthy subjects. Due to skewed distributions, the nonparametric Kruskal-Wallis test and Spearman's rank correlation coefficient were used.
3.3.2.2. Quantitative EEG calculations in study IV

QEEG was analyzed at the central frontal electrode (Fz) only. Theta frequency absolute power at this electrode was chosen based on our earlier research results (Study III), which show that CLO-caused changes are similar in all electrodes of frontal, central, and parietal regions and most specifically at Fz.

3.3.2.3. Quantitative EEG calculations in studies V and VI

The theta frequency band (3.5-7 Hz) in the fronto-central scalp area was chosen to be observed on the basis of the results of Study III, where we showed a widely distributed marked increase in the theta band with prominence in the fronto-central midline scalp area in the schizophrenic patients treated with CLO. For a greater accuracy the absolute theta powers of electrodes on fronto-central left (FCleft) (Fp1, F3, C3), midline (FCz) (FpZ, FZ, CZ), and right (FCright) (Fp2, F4, C4) scalp areas were averaged. The averaged absolute powers for FCleft, FCz and FCright were calculated for each patient separately and afterwards correlations with clinical parameters were calculated.

3.4 Clinical assessments

3.4.1 Positive and Negative Syndrome Scale

Positive and Negative Syndrome Scale (PANSS) is a 30-item rating instrument including 3 subscales: for positive symptoms (7 items), for negative symptoms (7 items), and for general symptoms (16 items). The scale is developed by Kay et al 1987 for evaluating the presence/absence and severity of psychopathology of the SCH. The PANSS was originally developed using the BPRS and the Psycho-pathology Rating Scale. All 30 items are rated on a 7-point scale (from 1=absent, to 7=extreme).
3.4.2. Liddle’s Factors

Liddle's proposed three factors (Liddle 1987) called reality distortion, disorganisation and psychomotor poverty. Liddle made a finer distinction between the two relatively independent dimensions within the positive symptom cluster (hallucinations and delusions) and the “disorganised” symptoms of disorganised speech, disorganised behaviour, and inappropriate affect (Arndt et al 1995).

Factors were derived from 14 PANSS items describing characteristic symptoms of each factor (reality distortion – P1, P3, P6; disorganisation – P2, G5, G9, G11; psychomotor poverty – N1, N2, N3, N4, N6, N7, G7). The items were chosen in collaboration with professor P.Liddle.

3.5 Statistical analysis

3.5.1. General

The study results are given in mean ± SD, or mean ± range. Differences were usually considered statistically significant for a priori hypothesis when the P value was less than 0.05, when multiple calculations were used, the P value was considered significant if less than 0.001. The SYSTAT Version 5 (1992) software was used for statistical calculations. The statistical methods used in the studies are presented as follows.

3.5.2. Statistics in studies I and II

For non-parametric data the Wilcoxon’s non-parametric test and Spearman’s correlation coefficient were used in the calculations in Study I and Study II. In Study II multivariate analysis of General Linear Modelling (Dillon and Goldstein 1984) was used in addition.
3.5.3. Statistics in study III

The nonparametric Kruskal–Wallis test and Spearman’s ranked correlation coefficient were used for comparing the mean absolute power values between the groups. In this study the significance was set at $P = 0.001$ due to multiple calculations.

3.5.4. Statistics in study IV

Mean values and Standard Deviations were calculated, Spearman’s correlations were used to correlate the means of changes in theta power value in the Fz electrode and the ROS production by MO. Multivariate analysis MGLH (Multivariate General Linear Hypothesis) and General Linear Modeling, with stepwise exclusion of dependent variables, was used.

3.5.5. Statistics in study V

Wilcoxon’s signed ranks test was used to check the differences between the baseline values and subsequent PANSS scores, and theta powers. Spearman’s correlation coefficient was used for calculation of correlations.

3.5.6. Statistics in study VI

Spearman's correlations were used for calculating correlations between QEEG powers and clinical parameters. An alpha level was set on 0.05. Multivariate analysis MGLH and General Linear Modelling were used to test the influence of age, sex, and anxiolytic medication.
4. ETHICS

All the recruited patients were started on CLO for clinical reasons. The protocols of all these studies comply with the laws and ethical standards of Finland and the Fifth Revision of the Declaration of Helsinki. The study protocol was authorised by the Ethics Committee of Tammiharju Hospital in Tammisaari. After complete description of the study, a written informed consent was obtained. Patients were recruited in whom a change of medication was considered appropriate on a clinical basis. The course of treatment was decided by the clinician responsible for treatment. Patients were able to withdraw their consent at any time.
5. RESULTS

5.1. Effect of clozapine on the production of reactive oxygen species by monocytes. Studies I and II

The ROS production was studied prospectively in eight patients for ten weeks. Although ROS production did not show significant longitudinal trends during the trial, the changes in ROS production by non-stimulated (MOn) and phorbol myristate acetate (PMA)-stimulated MO (MOs) at week 3 (values at base-line minus those at week 3) correlated positively with the changes on the total ($p=0.035$ and $p=0.009$, respectively) and negative ($p=0.045$ and $p=0.027$, respectively) Positive and Negative Syndrome Scale (PANSS) subscales. Production of ROM by MOs at week 10 correlated positively with PANSS positive subscale ($p=0.049$). A decrease or relatively small increase in the ROM production by MOn and MOs was associated with more favourable clinical outcome than a clear-cut increase in their ROS production. The serum concentrations of CLO at week 3 also correlated positively with the changes in ROS production by MOs at week 3 ($p=0.047$) and at week 10 ($p<0.001$).

The ROS production by MOn decreased after incubation *in vitro* with CLO at weeks 0 (baseline) and 3, with a similar trend seen also at week 10 of the trial. The ROS production decrease by MOn after CLO incubation *in vitro* at week 0 correlated positively with subsequent improvement in clinical symptomatology (PANSS total scores and subscales) at weeks 3 and 10 with exemptions of positive subscale at week 3 and negative subscale at week 10. The most significant correlation was found with PANSS negative subscale at week 3 ($p=0.003$).
5.2. EEG findings in patients taking conventional antipsychotics, clozapine and healthy subjects. Study III

The main difference between the CLO-treated group and the other groups was increased absolute theta power values. Statistical testing suggested significant differences \((P < 0.001)\) in the mean delta and theta power values on the FC between the CLO-treated and the non-CLO group as well as healthy controls. There was observed a wider range of absolute theta power values in the CLO-treated group compared with the healthy subjects and the non-CLO-treated group. There was no significant difference at any electrode in either the alpha power or the beta power distributions between the groups. There was no statistical difference in the delta, theta, alpha, or beta power values between the two hemispheres in any group.

5.3. The correlation of EEG findings with production of reactive oxygen species by monocytes. Study IV

The changes in the ROS production and theta power values of the Fz-electrode showed a clear-cut negative correlation after 3 weeks of CLO treatment \((p < 0.005)\) for non-stimulated and \((p < 0.001)\) stimulated MO. After 10 weeks of treatment there was no statistically significant correlation between these parameters.

The reduction of ROS production by MO after incubation with CLO \textit{in vitro} correlated positively with the increase in theta power after 3 weeks \((r =0.738, p<0.05)\), but after 10 weeks no correlation was observed.

The above correlations were examined further using multivariate stepwise analysis. The change in theta power of the Fz electrode was the dependent
variable and the change in ROS production, age, serum CLO level and duration of illness were independent variables. The increase of theta power in Fz was explained by the change in ROS production only: $R^2 = 0.929$, $p<0.05$ for non-stimulated, and $R^2 = 0.907$, $p<0.001$ for stimulated MO.

5.4. Theta power changes in patients receiving CLO treatment. Study V

After three, ten, and eighteen weeks of CLO treatment a clear-cut increase in theta power was observed in all electrode groups similarly, emerging most prominently in the midline. The increased theta power remained virtually unchanged after ten weeks of treatment.

After 3 weeks there were significant positive correlations between theta power increase in the midline and reduction in the PANSSP ($p<0.05$), and between theta power increase in all electrode groups and reduction in the PANSSN ($p<0.01$). After 10 weeks a significant correlation between theta power change in all electrode groups and changes in the PANSSG and PANSSP were observed ($p<0.05$). After 18 weeks some trend of inverse correlation between changes in the PANSSG and PANSSN and theta power change were observed, but not with regard to PANSSP. Mainly theta power change in FC left and right areas were found similar to midline.

A trend of positive correlation between change in PANSS and serum CLO levels was observed after three and ten weeks and a trend of inverse correlation after 18 weeks.
5.5. The correlation between different symptom clusters and the Quantitative EEG power spectrum. Study VI

Sixteen patients receiving different antipsychotic treatment were investigated. The hypothesis was postulated according to earlier research data of Harris et al (1999). Significant positive correlations were found between beta and psychomotor poverty (p<0.05) supporting the \textit{a priori} hypothesis. Trends to positive correlations (p<0.1) were observed between delta and PANSS negative subscale and Liddle’s psychomotor poverty. Alpha did not correlate with reality distortion and delta did not correlate with disorganisation.

\textit{Post hoc} analysis revealed positive correlations of the same magnitude between beta power and psychopathology generally over FC. Delta power showed positive correlation with PANSS negative subscale on the midline FC. MGLH testing for the influence of sex, age, and anxiolytic medication on the \textit{a priori} hypothesized correlation between beta power and psychomotor poverty on FCz revealed that age as a factor strengthened the correlations, other variables were rejected by the model: R2 0,828; p < 0,000.
6. DISCUSSION

6.1. Methodological aspects

This work can be considered as an attempt to clarify the mechanism of action of CLO and the ways forward in the development of biological markers for SCH and CLO treatment response. In the study it was found that CLO treated patients had marked slowing fronto-centro-parietally in the EEG which was associated with clinical improvement. Changes of ROS production by MO in patients treated with CLO seemed to predict the clinical improvement. The EEG findings were observed widely over the scalp indicating the involvement of multiple cortical areas in the brain related to neurophysiopathology of SCH. The results have been shown in patients suffering from chronic schizophrenia and experiencing relapse. Whether the results apply to SCH generally can not be answered on the basis of this study. It would also be fair to state that, following this study, the mechanism of action of CLO still remains obscure.

The study has some strengths as hospital inpatients were used, the adherence with medication was supervised, plasma concentrations for the CLO were monitored, and substance misuse during the study was highly unlikely. There was no concomitant antipsychotic treatment allowed with CLO. The patients who had had CLO treatment at any time in the past were excluded from the studies. The study was designed in an open setting although the data was analysed only after completion of the study.

The study has a number of limitations – a relatively small number of subjects, with only three patients not responding to CLO, a short wash-out period, no placebo, and no control group and due to the lack of control group the biological variation of ROS production by MO and EEG variation can not be taken into account. Other antipsychotic medication varied before initiation of CLO. A placebo response, especially at the beginning of CLO treatment, can not be ruled out as an explanation of the initial drug effect.
6.2 Effect of clozapine on reactive oxygen species production

There is currently evidence that ROS may play an important role in the pathophysiology of SCH. The elevated levels of free radicals and distorted balance between oxidants and antioxidants have been reported in patients with SCH (Dakhale et al 2004, Gama et al 2006). The atypical antipsychotics seem to modify activity of free radicals in CNS (Dakhale et al 2004). According to Metodiewa & Koska (2000) there are a number of reports suggesting the involvement of free radicals in a variety of pathological events and multistage disorders including neurotoxicity and apoptotic death as well as implications of involvement in Alzheimer's disease, Parkinson's disease and SCH.

In our study we found that CLO caused inhibition of production of ROS by blood MO in vitro predicted clinical improvement. We also found positive correlation between changes in ROS production by MO and clinical improvement. Our findings support the hypothesis of CLO having antipsychotic effect partly by modifying ROS production.

As peripheral blood MO may serve as a model for brain macrophages – microglia, the changes in ROS production might happen also in the brain, modulating neural processes and thus have a role in the pathophysiology of SCH. The reasons behind the superior antipsychotic efficacy of CLO over conventional antipsychotics in treatment of SCH remain obscure, but involvement of many mechanisms including neuroimmunological ones is one possible explanation. In our sample the patients were experiencing acute relapse and one might speculate that the ROS driven active neurotoxic processes were affected by CLO which inhibited in some cases further progression of the relapse.
6.3 EEG findings in patient with various antipsychotic medication, patients receiving clozapine and healthy subjects

Marked slowing in EEG caused by CLO was observed in this study. The increased amount of theta and delta activity was widespread but was emphasized in the frontal, central and parietal areas in the patients treated with CLO. The effect was detected in patients who were treated with CLO only or with CLO and with other antipsychotic drugs simultaneously. Patients treated with various other conventional antipsychotics did not differ significantly from healthy controls. The observed slowing of EEG readings during CLO treatment seems to reflect similar widely distributed changes of brain electrical activity.

Small et al. (1987) reported similar topographic EEG changes in theta amplitude during pure CLO treatment. The pharmacological mechanisms behind the slowing remain obscure, anticholinergic effect does not singularly account for this as patients taking various other antipsychotics with anticholinergic properties did not show EEG changes of the same magnitude. According to Small et al. (1987), chlorpromazine being a potent anticholinergic drug also slows EEG readings on the central and anterior scalp areas. In our work the slowing was not explained by chlorpromazine. Some patients had conventional antipsychotics in addition to CLO. Omitting of the eight patients who received CLO together with other antipsychotics, did not, however, affect the results.

The EEG changes observed in the fronto-centro-parietal scalp area might indicate functional change in the cortical areas of these regions as well as the reflection of the change in the functional activity of the limbic system. N-desmethylclozapine, the active metabolite of CLO potentially has partial agonistic properties similar to the partial D2 agonist aripiprazole (Burstein et al 2005) and thus it might selectively activate mesocortical dopaminergic pathways and inhibit mesolimbic ones as has been suggested in aripiprazole. In the rat,
CLO is known to affect neurotransmission preferentially in the mesocorticolimbic system, including the medial prefrontal cortex (Wang et al., 1994; Yamamoto et al., 1994). Morstyn et al. (1983) have reported both increased theta and delta on frontal areas, and increased beta on the left anterior temporal area in medicated patients with SCH compared with healthy control subjects. These differences are probably small compared with the remarkable slowing caused by CLO. In our study the healthy group and the group treated with other antipsychotics did not differ from each other except in delta power value at a single central occipital electrode (Oz).

Our results suggest widely distributed, marked EEG changes caused by and specific for CLO.

6.4 Reactive oxygen species production and EEG findings

In this study we investigated the correlation between inhibited ROS production by MO and increase of theta activity in eight patients receiving CLO. The increase of theta power showed negative correlation with ROS production by MO after three weeks of CLO treatment. It is possible that the finding reflects a direct simultaneous effect of CLO on the theta power and ROS production by MO. However, in the multivariate analysis the change in theta activity was better explained by change in ROS production than by serum CLO concentration.

One may speculate that EEG slowing is a result of the modulatory action of the activated microglia cells in CNS via production of ROS or cytokines or both. Microglia and activated microglia (brain macrophages) are able to produce ROS and the neurotoxic effect of activated microglia-produced ROS has been connected with diverse brain pathology including SCH (Banati et al 1993, Oken1995, Metodiewa 2000). MO may be used as a model for microglia, since
they share numerous functions, including production of ROS and cytokines (Langermans et al 1994, Chao et al 1995, Sacerdote at al 1995, Rock et al 2004). Cytokines have been reported to affect the production of biogenic amines in the CNS (Dunn et al 1995) and catecholamines (being biogenic amines) at the same time might regulate the cytokine response in CNS (Szelenyi & Vizi 2007). Biogenic amines have been shown to affect the EEG spectral power in the alert state as well as the EEG patterns during sleep (Crochet & Sakai 1999, Sebban et al 1999) in animal studies. In the present study this mechanism would provide an explanatory link between activation of microglia and slowing in the EEG.

The superior efficacy of CLO can possibly be explained by the effect of the drug on the brain physiology involving various neuromodulatory, immunological, and possibly metabolic processes. These processes might have an impact on neuroplasticity which is reflected also in the brain electrical activity. It also supports the hypothesis of possible involvement of neuropsychoimmunological mechanisms in the therapeutic effect of CLO.

6.5 Changes of theta power in patients receiving clozapine treatment

The sixteen patients started on the CLO treatment were prospectively studied to test the hypothesis of correlation between theta increase and therapeutic response. The hypothesis was raised on the basis of clinical observations. Prior to the present study we supposed that the absolute theta power increase preceded the clinical response to CLO treatment. However, the increase in theta power was not observed prior to the clinical response. The CLO induced theta power increase in FC emerged prominently almost two weeks later than the initial clinical response was observed after a week of CLO treatment. The marked theta power increase was observed three weeks after CLO was initiated and it correlated with clinical response after three and ten weeks of treatment. The
theta power increase was only minimal in those patients who did not respond to CLO.

Higher serum CLO concentrations seemed to be associated with better treatment responses in the beginning of the treatment, but not after ten and eighteen weeks. One reason for this may be that CLO, having been dosed according to clinical response, was overdosed in patients who responded poorly. While the correlations between treatment response and theta power increase were significant after three and ten weeks of treatment, there were no significant correlations between treatment response and serum CLO level. The change in theta frequency in the QEEG and particularly in the midline electrodes over the FC might be a more sensitive indicator for the evaluation of CLO treatment adequacy than the serum CLO level.

The explanation might be that theta power increase possibly reflects the receptor occupancy by CLO in the brain and the effect of the drug on brain function and is a better indicator of treatment adequacy than the CLO serum level. The mechanism of CLO induced theta increase is not completely understood, but along with the anticholinergic effect (Adler et al 2002) of CLO the influence on the many modulatory systems in the brain might be involved.

Our results indicated that midline EEG registration probably reflects reliably the clinically relevant changes in the EEG power spectrum related to SCH.

**6.6 The correlation between different symptom clusters and the EEG**

The correlation between symptom clusters and theta activity in patients evidencing relapse of SCH and responding inadequately to antipsychotic medication was investigated in this study. We postulated the *a priori* hypothesis according to the results of Harris et al (1999) where PANSSN score correlates
positively with delta power, Liddle's psychomotor poverty with delta and beta powers, disorganisation with delta power and reality distortion with alpha power. The finding of our study only partly supported the hypothesis - in the FCz significant correlation was found between beta absolute power and psychomotor poverty and the delta power showed trends towards positive correlation with PANSSN and Liddle's psychomotor poverty. We did not, however, observe correlation between delta and disorganisation and between alpha and reality distortion factors.

In supplementary analysis the correlations of the same magnitude, despite being *post hoc* findings, were seen between beta power and all clinical parameters over FC. The correlation between symptom clusters and absolute beta power indicated that the overall symptomatology correlates with absolute beta power. The correlation of beta power with a wide spectrum of symptoms was also proposed by Itil et al (1972). This finding supports the link between EEG background activity and severity of symptoms of SCH.

The psychiatric illness affects several mental functions simultaneously and, most probably affects the functioning of the whole neural network. The EEG background activity reflects the function of the neural network with good time resolution. Abundant beta activity in the EEG was probably the first report of EEG changes in patients with SCH. Davis (1940) reported “choppy activity” which probably referred to low voltage beta frequency. Earlier the increased beta activity has been suggested to be related to hyperarousal maintained by the brain stem (Sponheim et al 2000) and auditory hallucinations (Gaser et al 2004, Ropohl et al 2004, Lee et al 2006).
In patients with SCH the resting cortical electric activity is probably altered which is reflected in the EEG of the FC. Psychiatric symptoms are most likely a consequence of dysfunction of multiple cortical areas and subcortical brain structures in patients with SCH, which is also supported by findings of Gaser et al (2004). The QEEG remains a promising method for SCH research.
7. CONCLUSIONS

The main results and conclusions are:

I Patients treated with CLO showed widespread marked slowing in the QEEG compared to patients treated with other antipsychotics as well as healthy controls.

II After three and ten weeks of CLO treatment an association between ROS production by MO (in vivo) and clinical improvement was found. CLO inhibited ROS production by MOn (in vitro) at baseline which seem to predict the clinical response.

III Theta power increase in QEEG was associated with clinical improvement in CLO treated patients experiencing relapse of SCH and responding inadequately to treatment with conventional antipsychotics.

IV Changes in EEG activity in patients with SCH treated with CLO are diffusely located in the fronto-centro-parietal scalp area and are most robustly observed in the theta frequency band and culminate on the midline.

V Theta power increase in QEEG associated with CLO treatment was reflected by changes in ROS production by MO.

VI Beta power value is associated with negative symptoms and seems to correlate with overall psychopathology in patients evidencing relapse and responding inadequately to treatment with conventional antipsychotics.
8. FUTURE CONSIDERATIONS

In the future larger samples using narrower diagnostic criteria and applying long-term prospective research settings with control groups should be considered. The QEEG, used for investigating the brain background activity, seems to be a relevant and valid method for SCH research. It is worthwhile investigating the role of the ROS production by MO as a possible important neuropsychoimmunological mechanism in the pathogenesis of SCH.
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I know there are many others deserve to be personally acknowledged. I first would like to express my apologies and then to thank you all.

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Helsinki, November 2007

Andres Gross
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### 11. APPENDICES

#### 11.1. Appendix 1

**Table 1. Characteristics of patients and subjects studied.**

<table>
<thead>
<tr>
<th>Number of the study</th>
<th>Subjects</th>
<th>Number of subjects/patients</th>
<th>Mean age, in years</th>
<th>Sex M / F</th>
<th>Mean duration of illness, in years</th>
<th>Mean duration of medication, in years</th>
<th>Mean Chlorpromazine equivalents (mg)</th>
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<tr>
<td>Studies I, II, and IV</td>
<td>Patients, chronic SCH</td>
<td>8</td>
<td>31,4 (24 - 41)</td>
<td>1 / 7</td>
<td>9 (2 - 22)</td>
<td>8,9 (1 - 22)</td>
<td>516 (300 - 900)</td>
</tr>
<tr>
<td>Study III</td>
<td>Healthy subjects</td>
<td>29</td>
<td>35 (21 - 56)</td>
<td>8 / 21</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>&quot;=&quot;</td>
<td>Patients, chronic SCH, no CLO treatment</td>
<td>21</td>
<td>39 (28 - 67)</td>
<td>8 / 13</td>
<td>13,7 (4 - 41)</td>
<td>13,2 (1 - 37)</td>
<td>405 (80 - 1050)</td>
</tr>
<tr>
<td>&quot;=&quot;</td>
<td>Patients, chronic SCH and Schizoaff. Dis., CLO treatment,</td>
<td>21</td>
<td>37,6 (19 - 60)</td>
<td>13 / 8</td>
<td>15,7 (3 - 27)</td>
<td>15,5 (3 - 27)</td>
<td>514 (100 - 900) (CLO)</td>
</tr>
<tr>
<td>Studies V, VI</td>
<td>Chronic SCH</td>
<td>16</td>
<td>32,6 (22 - 43)</td>
<td>7 / 9</td>
<td>10,4 (2 - 26)</td>
<td>9,4 (1 - 23)</td>
<td>386 (0 - 900)</td>
</tr>
</tbody>
</table>
11.2 Appendix 2

Graph shows the location of electrodes according to The International 10-20 Electrode Placement System. Grey area indicates the fronto-central scalp area, referred in this study as FC.
12. ORIGININAL PUBLICATIONS I – VI, ATTACHED