Herpes viruses and neuroinflammation

Doorduin, J

Publication date:
2010

Chapter 1

General introduction

Schizophrenia

Schizophrenia is a chronic, severe and disabling brain disease that is characterized by abnormal mental function and disturbed behavior. At the beginning of the 20th century, the first comprehensive description of schizophrenia was provided by Emil Kraeplin when he called it dementia praecox [1]. He believed that schizophrenia was a degenerative disease that started out in the early childhood and would eventually lead to deterioration of personality and mind. Dementia praecox was renamed into schizophrenia by Eugen Bleuler [2] when he realized that the disorder did not necessarily lead to mental decline and did not always occur in young people. Schizophrenia literally means 'split mind', referring to the loss of capacity to guide thought processes by concepts that were correctly linked together. The term schizophrenia is, however, still controversial since it often leads to misinterpretation of the disease as being a multiple personality disorder.

Approximately 1% of the human population world-wide is affected by schizophrenia [3] and besides the disorder being devastating for most patients, it is very costly for families and society. The age of onset of schizophrenia is often between 16 and 25 years of age, and rarely before puberty or after 40 years of age. Diagnosis is made according to the appearance of positive and negative symptoms [4]. Symptoms are referred to as positive when they represent abnormal behavior, such as hallucinations, delusions and unorganized thinking. The positive symptoms of schizophrenia are characteristic of psychosis and appear in episodes of time. The negative symptoms refer to the absence of normal behavior, resulting in, amongst others, social withdrawal, flattened emotion and the lack of spontaneous thinking. In addition to the positive and negative symptoms, schizophrenic patients show cognitive symptoms, with impairments of attention, memory and executive functions, as well as mood symptoms, such as suicidality and hopelessness.

In general, schizophrenic patients are treated with antipsychotic drugs to control the symptoms. The first generation of antipsychotics, the so-called typical antipsychotics, were only effective in controlling the positive symptoms of schizophrenia. However, the second generation of atypical antipsychotics were also found to control the negative and cognitive symptoms. Although in many of the schizophrenic patients the symptoms are successfully treated, the cure for schizophrenia has not yet been found. Perhaps one of the most important reasons for the lack of a cure for schizophrenia is that the etiology of the disease is still unknown.

Although the etiology of schizophrenia is not known, many structural and functional brain abnormalities have been found. The main structural abnormalities include a reduction in grey matter volume, mainly in the prefrontal and temporal brain regions, and/or an increase in ventricular volume [5]. In addition, the connections between neurons are thought to be altered in schizophrenia, which was measured post-mortem [6], but also with diffusion tensor imaging [7]. Altered neuronal connections, may consequently lead to changes in neurotransmission. The neurotransmitter that was originally thought to underlie the etiology of schizophrenia was dopamine. This was mainly based on the finding that drugs that increase dopamine in the brain, such as amphetamines and cocaine, cause psychotic symptoms. In addition, it was accidently discovered that drugs that antagonized the binding of dopamine caused a decrease in psychotic symptoms. It was later shown that the neurotransmitter glutamate is also involved in schizophrenia. Glutamatergic dysfunction is thought to be related to a hypofunction of the glutamate NMDA-receptor, since the NMDA-antagonists ketamine, phencyclidine (PCP) and dizocilpine (MK-801) were found to induce a condition that resembles schizophrenia. Since both dopamine and glutamate play a role in schizophrenia it is most likely that they interact in inducing schizophrenia, involving also GABAergic and cholinergic systems [8].

Although the findings mentioned before indicate abnormalities in the schizophrenic brain, they do not explain the etiology per se. Both gene mutations and various environmental factors have been suggested to play a role in the etiology of schizophrenia, but neither of these single factors can explain the entire disease process. It is therefore generally agreed that environmental factors underlie the development of schizophrenia only in genetically predisposed individuals. When focusing on environmental factors, one could think of factors such as substance abuse and stressful life events, but also infectious agents are thought to play an important role in the etiology of schizophrenia.

Kraeplin and Bleuler [1,2] were one of the first to propose that infectious agents might play a role in schizophrenia and its development. After half a century of silence, the interest in the infectious hypothesis of schizophrenia revived [9]. Early studies showed a winter and spring seasonality of birth of individuals who developed schizophrenia later in life, which was, amongst others, suggested to be caused by infectious agents [10,11]. Indeed, a significant correlation was found between schizophrenic births and the occurrence of measles, Varicella-Zoster virus and polio [12], as well as influenza [13], suggesting the role of infectious agents in the

development of schizophrenia. In addition, it has also been shown that serious viral infections of the brain during childhood were associated with later development of schizophrenia [14]. These studies indirectly provided evidence for the involvement of infectious agents in schizophrenia and are just a small selection of all the work done.

The infectious agents that are most likely to be involved in schizophrenia are viruses, which show preference for infecting the central nervous system and have the ability to establish latency in the human body. If viruses (or other infectious agents) are proven to play a role in schizophrenia, this can lead to improved treatment and thus better treatment outcome. It is therefore of great value to provide more evidence for the involvement of viruses in schizophrenia, which can, amongst others, be obtained by determining antibodies in serum and cerebrospinal fluid, as well as antigens in the central nervous system. Since most of these studies focused on herpes viruses, only these viruses and their role in schizophrenia will be discussed in more detail.

Herpes viruses in schizophrenia

Herpes viruses are a family of DNA viruses, of which 8 types are known to infect humans. These include the herpes simplex virus type-1 (HSV-1), herpes simplex virus type-2 (HSV-2), Varicella-Zoster virus (VZV), Epstein Barr virus (EBV), cytomegalovirus (CMV) and the human herpes viruses 6, 7 and 8 (HHV 6-8). Herpes viruses are large, enveloped viruses that contain double-stranded DNA, surrounded by an icosadeltahedral capsid [15] (figure 1). The viral envelope contains many glycoproteins that are used for viral attachment, fusion and for escaping immune control. The replication of herpes viruses is initiated when viral glycoproteins interact with cell surface receptors. Following interaction, the capsid is released into the cell and delivers the DNA into the nucleus, where it is transcribed and replicated. Transcription of herpes viruses is regulated by both viral and cellular nuclear factors, which determine whether the infection is lytic, persistent or latent. The ability of herpes viruses to establish latency in the human body makes them attractive candidates for a role in schizophrenia. Primary infection with the majority of the herpes viruses mainly occurs during childhood, without the appearance of clinical symptoms. Periodical reactivation of the viruses later in life could explain the episodes of positive symptoms (psychosis) that schizophrenic patients experience.

Evidence for the role of viruses in schizophrenia can be obtained by measuring viral antibodies in serum and cerebrospinal fluid in schizophrenic patients. Over twenty

studies investigated antibodies against several herpes viruses, but were inconclusive, since both negative and positive associations between schizophrenia and herpes viruses were found (reviewed in [9]). A more direct approach is to study the viral presence in the schizophrenic brain. However, studies on the herpes virus genome in the post-mortem schizophrenic patient predominately showed negative results [9,16]. In one study it has clearly been shown that HSV-1 is present in the schizophrenic brain, but this was not found to be different in comparison with healthy controls. The problem, however, with post-mortem brain research is that the virus could have been present in brain areas that were not tested, that the viral genome cannot be detected by the techniques used or that the virus is latently present at the time of death.
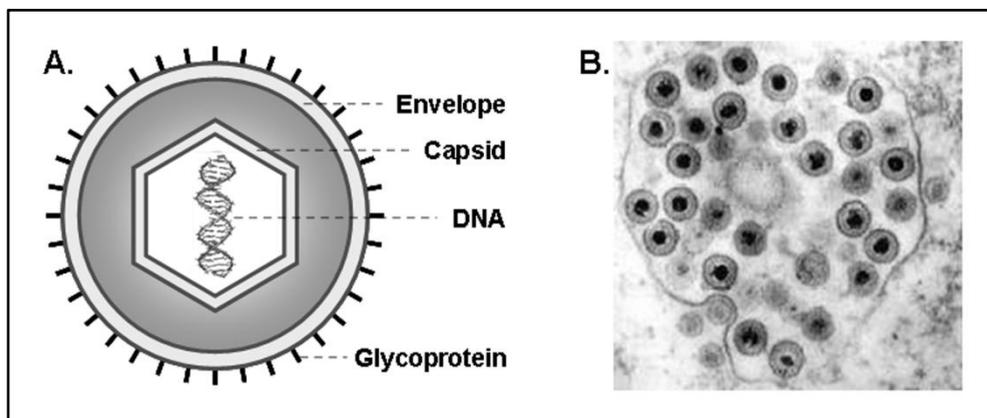

Figure 1 Herpes viruses. **A.** Structure of the herpes virion. **B.** Thin section of virions as they leave the nucleus of an infected cell (magnification of approximately 40.000x); micrograph from F. A. Murphy, School of Veterinary Medicine, University of California, Davis, USA.

More convincing evidence for the role of herpes viruses is provided by studies which showed associations between serum antibodies against herpes viruses and brain abnormalities or disturbances. In MRI studies, it was found that schizophrenic patients that were seropositive for HSV-1 had increased cortical atrophy when compared to seronegative patients [17], and that HSV-1 seropositive first-episode schizophrenic patients had a significant reduction in prefrontal grey matter volume, when compared to seropositive healthy controls and seronegative first-episode schizophrenic patients [18]. In addition, it has been shown that HSV-1 seropositive schizophrenic patients had a lower cognitive functioning than seronegative patients, as

measured by the neuropsychological status of the patients [19]. Seropositivity for other herpes virus, including HSV-2, CMV, EBV and VZV, was not associated with cognitive functioning. In contrast, it was shown by Shirts *et al.* [20] that not only schizophrenic patients that were seropositive for HSV-1, but also CMV seropositive patients showed impaired cognitive function, when compared to seronegative patients, as measured by visual conceptual and visuo-motor tracking. When it was investigated if the anti-herpes virus drug valacyclovir could reduce symptoms in schizophrenic patients, a significant improvement in the total score on the PANNS was found, in CMV seropositive patients [21]. Improvement was not found in patients that were seropositive for other herpes viruses. Taken together, these studies suggest that herpes viruses in schizophrenic patients could, in part, be responsible for the found brain abnormalities, deficits in cognitive functioning and symptoms.

There is thus evidence suggesting that herpes viruses are involved in schizophrenia, however, additional research is necessary to further unravel the role of herpes virus in (the etiology of) schizophrenia. Although herpes viruses may be involved in schizophrenia, a possible involvement of immune mechanisms in schizophrenia has, in addition, been proposed.

Immune mechanisms and neuroinflammation in schizophrenia

The immune system functions as a defense of the body against infectious agents, to prevent disease development. There are two components of the immune system, being the innate and adaptive immune systems. The innate immune system provides an immediate but non-specific response, involving macrophages, granulocytes and natural killer cells. A stronger immune response is provided by the adaptive immune system, characterized by B- and T-lymphocytes, and has an immunological memory. Important signaling molecules for both the innate and adaptive immune systems are cytokines, which can have anti-inflammatory or pro-inflammatory properties. Mainly based on the cytokines expressed, the T-helper lymphocytes are classified into type-1 and type-2, which work together in the immune response. Schizophrenia has been associated with an increase of cytokines in serum and cerebrospinal fluid [22], suggesting the presence of an inflammatory process. In addition, it has been proposed that an imbalance between the type-1 and type-2 immune response is involved in schizophrenia [23]. This imbalance is suggested to indirectly affect dopaminergic and glutamatergic neurotransmission.

In the brain, the innate immune system is represented by microglia cells, which provide the first line of defense against infectious agents or injury, resulting in neuroinflammation. Microglia cells are thought to be derived from monocytes in bone marrow [24]. In the healthy adult brain, microglia cells have a ramified morphology, characterized by a small body with long processes that are continuously used to survey the microenvironment. The morphology of the microglia cells changes into a reactive and amoeboid form when the brain is infected or injured, in response to signals from damaged neurons. Transformation of resting into activated microglia cells is a rapid process and can be divided into two stages. The first stage is characterized by non-phagocytic activated microglia cells, while in the second stage the microglia cells become phagocytic brain macrophages [25]. Activated microglia can express a variety of neurotrophic, anti-inflammatory molecules as well as neurotoxic, pro-inflammatory molecules, such as cytokines, depending on the stage of activation. It remains to be elucidated which factors determine if microglia cells express neurotrophic or neurotoxic molecules, but it has been proposed that in response to acute injury, the microglia cells express neurotrophic molecules, whereas neurotoxic molecules are expressed in chronic disease [26]. Although microglia cells play an important role in neuroinflammation, they do not act alone. Astrocytes are also activated in response to brain injury. The molecules expressed by the activated microglia cells and astrocytes recruit T-lymphocytes and macrophages from the periphery, which are also important for the neuroinflammatory process.

Neuroinflammation was found to be a feature of many neurological disorders, such as Parkinson's disease, Alzheimer's disease, multiple sclerosis and viral encephalitis, as measured in the post-mortem brain and by non-invasive imaging techniques. In addition, neuroinflammation was also implicated in schizophrenia. Studies that were performed to determine if neuroinflammation was present in the schizophrenic brain have mainly been focused on the detection of microglia cells in the postmortem brain. These studies gave conflicting results, since some studies report an increase in the presence of activated microglia cells in the schizophrenic brain [27,28], when compared to the healthy brain, while others could not find such an increase [29,30]. The discrepancy could be due to a variety of factors, like the methods used, the selection of brain areas and the type of schizophrenic patients that were studied. Related to the type of studied schizophrenic patients, it has been shown that activated microglia cells were only found to be present in the brain of schizophrenic patients that committed suicide during acute psychosis [31].

In addition to the postmortem studies, an increased activation of microglia cells in recent-onset schizophrenic patients has been shown by positron emission tomography (PET) imaging [32] with [11C]-PK11195. Logically, the main advantage of PET is that the presence of activated microglia cells can be studied in living schizophrenic patients, at all stages of disease.

Positron emission tomography

Positron emission tomography (PET) is a non-invasive imaging technique used to study functional processes in the body. The principle of PET is based on the coincidence detection of two gamma rays emitted by radioactive isotopes, which are attached to a particular biologically active molecule (figure 2).
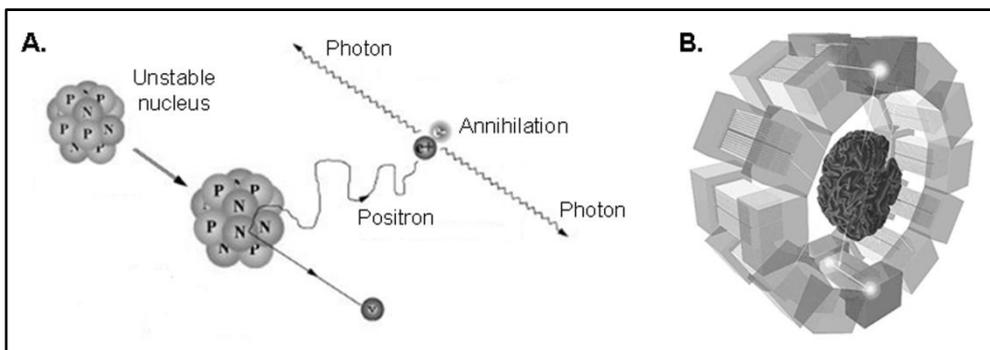

Figure 2 The principle of positron emission tomography. **A.** Positron emission by an unstable nucleus, followed by annihilation with an electron. Annihilation results in two photons that travel in opposite direction. Adapted from: http://depts.washington.edu/nucmed/IRL/pet_intro/intro_src/section2.html. **B.** Coincidence detection of photons by the detectors in the PET camera. From: http://neurocenter.unige.ch/groups/zaidi.php.

The radioactive isotopes used for PET are positron emitters. Positron emitters are isotopes that have an unstable nucleus, which decays by the conversion of a proton into a neutron, during which a positron is emitted. A positron has the same mass as an electron, but has a positive charge instead of the negative charge of electrons and is thus the antiparticle of an electron. Because of the abundance of electrons, the emitted positron will soon meet with an electron. When a positron has lost most of its kinetic energy and meets an electron, they annihilate and the total mass of both particles is

converted into energy according to Einstein's formula $E=mc^2$. The energy is emitted as a pair of photons of 511 keV each, that travel in opposite direction from the site of annihilation.

Most radioactive isotopes that are used in PET are produced by a cyclotron. In the cyclotron, a proton or deuteron beam is accelerated within a magnetic field to gain enough energy before it is ejected to target molecules. When the proton hits the nucleus of the target atom, radioactive isotopes are formed. Depending on which target atom used, different isotopes can be produced. Historically, the most commonly used isotopes for PET are ^{15}O, ^{13}N, ^{11}C and ^{18}F. The half-life of these isotopes, i.e. the time in which half of the radioactivity decays, are 2 min, 10 min, 20 min and 110 min, respectively. Nowadays, other isotopes are applied as well, such as ^{68}Ga, ^{82}Rb, ^{89}Zr, ^{124}I and ^{64}Cu. The radioactive isotope is used for chemical synthesis of the radiotracer. The radiotracer is then the biologically active molecule of interest, for studying a particular process in the body, labeled with a radioactive isotope.

After the radiotracer is injected, it will take part in the biological process of interest and the decay of the radioactivity can be imaged by a PET camera. The PET camera consists of a ring of detectors that can detect the two photons by coincidence detection. Only when two photons are detected within a short time-window by detectors that are approximately at opposite positions, they are considered to be coincident. All coincidence detections are assigned to a line of response, enabling calculation of the position of annihilations. All the annihilations together are used to create a 3D image in which the functional process is visualized. The images can be used for visual examination of, for example, abnormalities or for quantification of the functional process by use of pharmacokinetic models. Because PET is an attractive technique for studying functional processes within the body, it is widely used for both clinical and research purposes, of which the latter also involves animal studies using dedicated small animal PET cameras.

Aim and outline of the thesis

Driven by the viral hypothesis of schizophrenia, the aim was to further study the role of herpes viruses in schizophrenia. Therefore, three different goals were postulated:

1. To study the behavioral and functional consequences of herpes virus infections in rats;
2. To determine if neuroinflammation is present in the brain of schizophrenic patients;
3. To determine if active herpes viruses could be detected in the schizophrenic brain.

The major tool that was used to achieve these goals was PET, since this technique can be used to non-invasively study functional processes in the brain.

Chapter 2 to chapter 5 describe the validation of PET as a tool for imaging of neuroinflammation and active herpes viruses in the brain. *Chapter 2* reviews the use of PET for imaging of neuroinflammation, with a particular focus on the peripheral benzodiazepine receptors as the target for disease and therapy monitoring. In *chapter 3* it was evaluated if PET could be used to study neuroinflammation and active herpes viruses in the rat brain, with [^{11}C]-PK11195 and [^{18}F]-FHBG, respectively. *Chapter 4* and *chapter 5* describe the evaluation of three potential new PET tracers for imaging of neuroinflammation in rats, since the only PET tracer validated for clinical studies, [^{11}C]-PK11195, may not be sensitive enough to visualize mild neuroinflammation.

In chapter 6 to chapter 8, the behavioral and functional consequences of herpes virus infection of the rat brain were described. *Chapter 6* describes the possible link between herpes virus infection, neuroinflammation and neurotransmitters. Antipsychotic treatment in herpes virus infected rats was evaluated in *chapter 7*. In *chapter 8* the possible link between herpes virus induced neuroinflammation and treatment resistance in schizophrenia was studied.

The human PET studies are described in chapter 9 to chapter 11. In *chapter 9* it was studied if neuroinflammation was present in schizophrenic patients and in *chapter 10* it was determined if active herpes viruses could be detected in the schizophrenic brain. In *chapter 11* the role of PET in unraveling the role of herpes viruses in schizophrenia was discussed.

In the final chapters the thesis is summarized in *chapter 12* and *chapter* 13 describes the future perspectives, with concluding remarks. A Dutch version of the summary is provided in *chapter 14*.

References

1   Kraeplin, E. and Barclay, R. M. Dementia praecox and paraphrenia. Edinburgh: E and S Livingstone, 1919

2   Bleuler, E. Dementia praecox or the group of schizophrenias. New York: International Universities Press, 1911

3   World Health Organization. Neurological disorders; public health challenges.: WHO, 2006

4   Lewis DA, Lieberman JA. Catching up on schizophrenia: natural history and neurobiology. Neuron 2000; 28:325-334

5   Davatzikos C, Shen D, Gur RC, et al. Whole-brain morphometric study of schizophrenia revealing a spatially complex set of focal abnormalities. Arch.Gen.Psychiatry 2005; 62:1218-1227

6   Benes FM. Emerging principles of altered neural circuitry in schizophrenia. Brain Res.Brain Res.Rev. 2000; 31:251-269

7   Kyriakopoulos M, Bargiotas T, Barker GJ, Frangou S. Diffusion tensor imaging in schizophrenia. Eur.Psychiatry 2008; 23:255-273

8   Lisman JE, Coyle JT, Green RW, et al. Circuit-based framework for understanding neurotransmitter and risk gene interactions in schizophrenia. Trends Neurosci. 2008; 31:234-242

9   Yolken RH, Torrey EF. Viruses, schizophrenia, and bipolar disorder. Clin.Microbiol.Rev. 1995; 8:131-145

10 Torrey EF, Torrey BB, Peterson MR. Seasonality of schizophrenic births in the United States. Arch.Gen.Psychiatry 1977; 34:1065-1070

11 Torrey EF, Bowler AE, Rawlings R, Terrazas A. Seasonality of schizophrenia and stillbirths. Schizophr.Bull. 1993; 19:557-562

12 Torrey EF, Rawlings R, Waldman IN. Schizophrenic births and viral diseases in two states. Schizophr.Res. 1988; 1:73-77

13 Mednick SA, Machon RA, Huttunen MO, Bonett D. Adult schizophrenia following prenatal exposure to an influenza epidemic. Arch.Gen.Psychiatry 1988; 45:189-192

14 Dalman C, Allebeck P, Gunnell D, et al. Infections in the CNS during childhood and the risk of subsequent psychotic illness: a cohort study of more than one million Swedish subjects. Am.J.Psychiatry 2008; 165:59-65

15 Murray, P. K., Rosenthal, K. S., Kobayasi, G. S., and Pfaller, M. A. Medical Microbiology. St. Louis: C.V. Mosby, 2002

16 Stevens JR, Langloss JM, Albrecht P, Yolken R, Wang YN. A search for cytomegalovirus and herpes viral antigen in brains of schizophrenic patients. Arch.Gen.Psychiatry 1984; 41:795-801

Ch. 1

17 Pandurangi AK, Pelonero AL, Nadel L, Calabrese VP. Brain structure changes in schizophrenics with high serum titers of antibodies to herpes virus. Schizophr.Res. 1994; 11:245-250

18 Prasad KM, Shirts BH, Yolken RH, Keshavan MS, Nimgaonkar VL. Brain morphological changes associated with exposure to HSV1 in first-episode schizophrenia. Mol.Psychiatry 2007; 12:105-113

19 Dickerson FB, Boronow JJ, Stallings C, Origoni AE, Ruslanova I, Yolken RH. Association of serum antibodies to herpes simplex virus 1 with cognitive deficits in individuals with schizophrenia. Arch.Gen.Psychiatry 2003; 60:466-472

20 Shirts BH, Prasad KM, Pogue-Geile MF, Dickerson F, Yolken R, Nimgaonkar VL. Antibodies to cytomegalovirus and Herpes Simplex Virus 1 associated with cognitive function in schizophrenia. Schizophr.Res. 2008; 106:268-274

21 Dickerson FB, Boronow JJ, Stallings CR, Origoni AE, Yolken RH. Reduction of symptoms by valacyclovir in cytomegalovirus-seropositive individuals with schizophrenia. Am.J.Psychiatry 2003; 160:2234-2236

22 Potvin S, Stip E, Sepehry AA, Gendron A, Bah R, Kouassi E. Inflammatory cytokine alterations in schizophrenia: a systematic quantitative review. Biol.Psychiatry 2008; 63:801-808

23 Muller N, Schwarz MJ. A psychoneuroimmunological perspective to Emil Kraepelins dichotomy: schizophrenia and major depression as inflammatory CNS disorders. Eur.Arch.Psychiatry Clin.Neurosci. 2008; 258:97-106

24 Ferrer I, Bernet E, Soriano E, del RT, Fonseca M. Naturally occurring cell death in the cerebral cortex of the rat and removal of dead cells by transitory phagocytes. Neuroscience 1990; 39:451-458

25 Kreutzberg GW. Microglia: a sensor for pathological events in the CNS. Trends Neurosci. 1996; 19:312-318

26 Nakajima K, Kohsaka S. Microglia: neuroprotective and neurotrophic cells in the central nervous system. Curr.Drug Targets Cardiovasc.Haematol.Disord. 2004; 4:65-84

27 Bayer TA, Buslei R, Havas L, Falkai P. Evidence for activation of microglia in patients with psychiatric illnesses. Neurosci.Lett. 1999; 271:126-128

28 Radewicz K, Garey LJ, Gentleman SM, Reynolds R. Increase in HLA-DR immunoreactive microglia in frontal and temporal cortex of chronic schizophrenics. J.Neuropathol.Exp.Neurol. 2000; 59:137-150

29 Steiner J, Mawrin C, Ziegeler A, et al. Distribution of HLA-DR-positive microglia in schizophrenia reflects impaired cerebral lateralization. Acta Neuropathol. 2006; 112:305-316

30 Wierzba-Bobrowicz T, Lewandowska E, Lechowicz W, Stepien T, Pasennik E. Quantitative analysis of activated microglia, ramified and damage of processes in the frontal and temporal lobes of chronic schizophrenics. Folia Neuropathol. 2005; 43:81-89

31 Steiner J, Bielau H, Brisch R, et al. Immunological aspects in the neurobiology of suicide: elevated microglial density in schizophrenia and depression is associated with suicide. J.Psychiatr.Res. 2008; 42:151-157

32 van Berckel BN, Bossong MG, Boellaard R, et al. Microglia Activation in Recent-Onset Schizophrenia: A Quantitative (R)-[(11)C]PK11195 Positron Emission Tomography Study. Biol.Psychiatry 2008; 64:820-822