Neonatal viral brain infection and the development of prepulse inhibition: A neurodevelopmental model of schizophrenia

Pro gradu

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Epidemiological data suggest an important role of perinatal viral infections in the etiology of schizophrenia. In this thesis the connection between neonatal viral brain infection and its consequences to the development of central nervous system was studied.

In schizophrenia the symptoms are divided into three categories as positive, negative and cognitive ones. Positive symptoms refer to hallucinations and delusions, negative symptoms are defined as social withdrawal, apathy and poor motivation and cognitive symptoms include deficits in abstraction and paying attention into subjects. Symptoms suggest that in schizophrenia the received information can not be filtered properly in central nervous system, but comes into patients senses in excess i.e. there are defects in sensorimotor gating.

Sensorimotor gating was studied by prepulse inhibition of acoustic startle -phenomenon. Prepulse inhibition refers to the inhibition of the startle reflex by weak prepulse presented before the startling stimulus. In schizophrenic patients prepulse inhibition is decreased and in addition to that psychotomimetic drugs disrupt prepulse inhibition in humans as well as in experimental animals. Sensorimotor gating ability is developed under neuronal development and it can be affected by several neurodevelopmental disturbances. In the present study rats were infected with herpes simplex type 1 virus at neonatal age and later challenged to dopaminergic and glutamatergic systems. Results show controversial data of effects on prepulse inhibition, still some attenuation can be seen. Challenge studies did not show clear and persistent effect either in dopaminergic or glutamatergic tests.

Corticosterone, naturally occurring hormone in rats, was administered to rat mothers under gestation until weaning in terms to clarify its effects to neuronal development. Administration was carried out by implanted pellet as well as by drinking water. The latter was found to work out better as it releases corticosterone in pulsatile manner. Corticosterone was administered also in acute test to drug naïve animals. This test showed significant decrease on prepulse inhibition. The same could not be repeated in corticosterone challenge test after perinatal treatments.

Nitric oxide synthase inhibitor L-NMMA was administered to neonates under days 5-9 after partus. This was supposed to prevent neonates from neurodevelopmental disturbances affected by virus and corticosterone. Despite various dose levels used, any clear effect could not be seen.

In summary, the studies show some effect of treatments on neuronal development and sensorimotor gating measured by prepulse inhibition. In the test groups inspected many treatments showed effect at first, but those effects disappeared at later tests as rats grew up. This might be an outcome of the potential compensatory mechanisms of the central nervous system to counteract harmful neurodevelopmental events.

Avainsanat – Nyckelord
schizophrenia; prepulse inhibition; herpes simplex virus; perinatal viral infection

Säilytyspaikka – Förvaringställe
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Abbreviations

AMP	amphetamine
ASR	acoustic startle reflex
BDV	Borna disease virus
CORT	corticosterone
CTR	control recording
DA	dopamine
EPS	extrapyramidal symptoms
GABA	gamma-aminobutyric acid
HSV	herpes simplex virus
5-HT	5-hydroxytryptamine
LCMV	Lymphocytic choriomeningitis virus
L-NMMA	N^G-methyl-L-arginine
NA	nucleus accumbens
NMDA	N-methyl-D-aspartate
PCP	phencyclidine
PFU	plague forming units
PPI	prepulse inhibition
RCMV	rat cytomegalovirus
s.c.	subcutaneous
VTA	ventral tegmental area

1. Introduction

The history of understanding schizophrenia as a psychiatric disorder has changed as a hundred years have passed. Not long ago this illness was thought to be of social origin and connected to upbringing of children. Something which has laid suspicion and self-accusations over parents and societies has taken completely different direction as a neurobiological disorder.

A schizophrenic patient is disabled by attenuation of reality, but before the situation stands in this point a lot has happened in the central nervous system (CNS). A developing brain can be affected by various environmental factors either in uterus or after birth. By changing the course of a normal neuronal development, these factors might pronounce malfunctions added with later disturbances in individuals life. These factors taken together lead into the first symptoms of schizophrenia.

2. Schizophrenia

2.1 Definition and symptoms

Schizophrenia is a disease with abnormal behavior and defects of thinking which apparently ruin individual's ability to perform tasks considered in normal life. Patients show psychotic

symptoms which means their sense of reality is heavily attenuated. Diverse characteristics of schizophrenia are divided into three classes of symptoms: positive, negative and cognitive. When coming to the definition of schizophrenia, these symptoms have been going on for several months *i.e.* the disorder has taken chronic features. Positive symptoms refer to delusions, hallucinations and odd thoughts. Negative symptoms are recognized as social withdrawal, apathy and poor motivation. Cognitive symptoms include deficits in abstraction, verbal memory and attention and are seen as the most difficult ones to treat (Breier 1999; Sawa and Snyder 2002).

2.2. Etiology and epidemiology

Despite the large body of research done, the clear mechanism and origin of schizophrenia is still waiting to be uncovered. Epidemiological studies have pointed out many important factors to be studied more closely and made some good guidelines where to head next.

Incidence of schizophrenia is approximately 1 % in entire population and in this figure there is no need for sorting out different nations or races. When zooming to the social classes, no differences at the rate of schizophrenia incidence can be distinguished (Yolken and Torrey 1995). However, in prevalence between social classes the situation is different, because the social status of schizophrenia patients tend to lower in terms of unemployment etc. (Kohn 1968).

Gender-related differences can be seen at the age of onset of the disease. Men usually develop schizophrenia at the age of 15-25, women at 25-35. However, prevalence of the disorder is equal in both sexes (Tamminga 1997).

Epidemiological and genetical evidence shows that schizophrenia is based on genes. The closer relatives have got the disorder, the more risk there is for offspring to develop it as expressed in figure 2-1 (McGuffin 1995). Risk factor does not reach 100 per cent even in the case of monotzygotic twins, which share all genes. This has led into thinking that there should be environmental factors affecting the development of schizophrenia.

2.3 Neurotransmitters and treatment of schizophrenia

During the 20th century the understanding of neuronal communication has evolved strongly and simultaneously the neurochemical models of schizophrenia have been introduced. Neurotransmitters dopamine (DA), glutamate, 5-hydroxytryptamine (5-HT) and gamma-aminobutyric acid (GABA) are mostly studied in respect of schizophrenia.

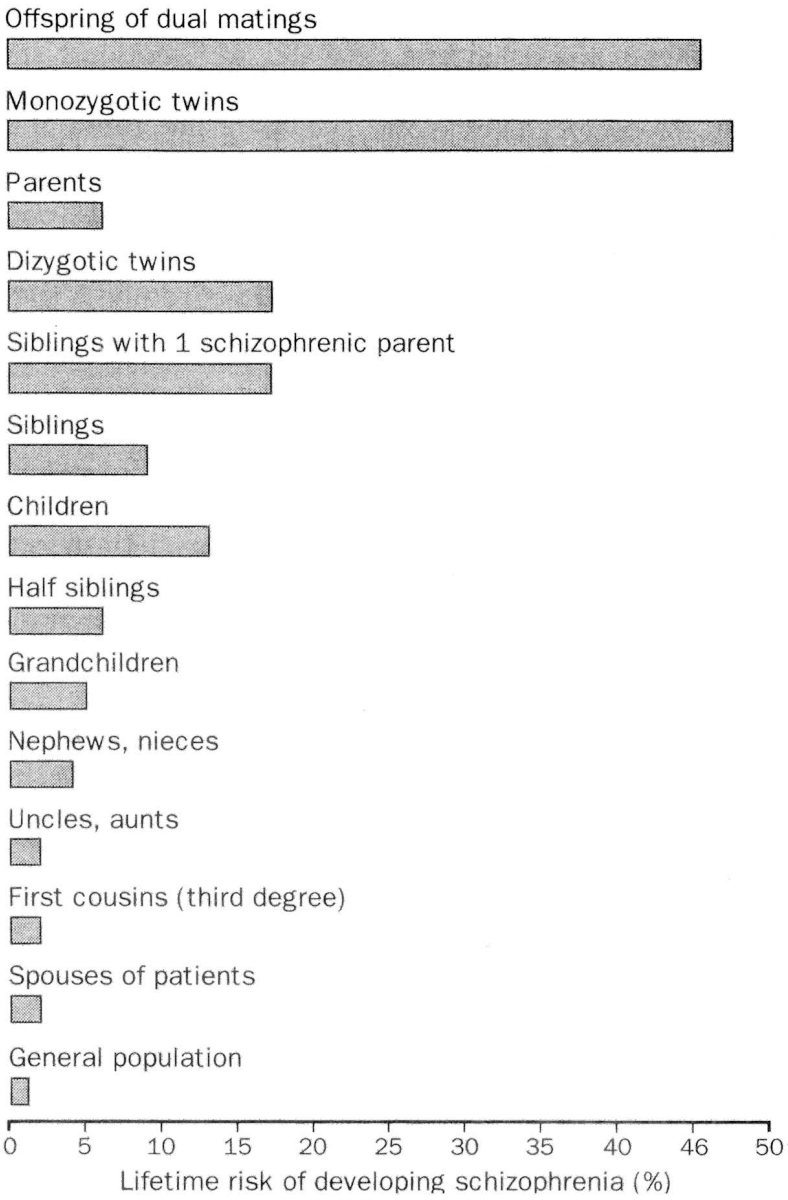

Figure 2-1: **Average risks for developing schizophrenia**

European studies 1920-1987 (McGuffin *et al.* 1995)

2.3.1 Dopamine

DA has been recognized to be involved in the pathophysiology of schizophrenia for a long time. DA hypothesis of schizophrenia is based on the research which claim overactive DA system to induce schizophrenic symptoms (Hietala *et al.* 1995). This overactivity of DA is located in ventral tegmental area (VTA) of the midbrain, *nucleus accumbens* (NA) and limbic cortex (Lieberman *et al.* 1997). There are two supporting points for DA hypothesis. Firstly, antipsychotic drugs blocking DA receptors have been shown to relief symptoms in schizophrenia in dose-response-manner. The binding affinity of these drugs for the D_2-receptor is related to clinical outcome of the symptoms. Secondly, DA agonists like amphetamine (AMP) are able to induce psychotic symptoms to healthy humans and on the other hand exacerbate symptoms of schizophrenia patients. AMP (figure 2) acts by releasing DA from presynaptically situated vesicles (Carlsson 1988).

2.3.2 Glutamate

There are certain major issues that emerge in speculations concerning DA-hypothesis of schizophrenia. New antipsychotic drugs accepted to treatment rationale of the disease do not bind as strongly to D_2-receptors as the older ones, but still exhibit better clinical effects (Kerwin 1994). The effect has to be mediated via other receptors, as is the case. Other neurotransmitters than DA have been found to be involved in schizophrenia in studies which

have shown that drugs affecting glutamate and 5-HT systems of neurotransmission are capable of pronouncing schizophrenia-like symptoms (Tamminga 1999). An observation in schizophrenia is phencyclidine (PCP) -related induction of psychotic symptoms to humans. PCP is a ligand of the N-methyl-D-aspartate (NMDA) receptor and this effect is carried out by blocking the receptor. Other known antagonists for NMDA-receptor, which also possess psychotomimetic properties, are research-used substance dizocilpine (MK-801) and clinically administered drug ketamine (Sawa and Snyder 2002). Structural formulas of drugs affecting for NMDA receptor are shown in figure 2-2.

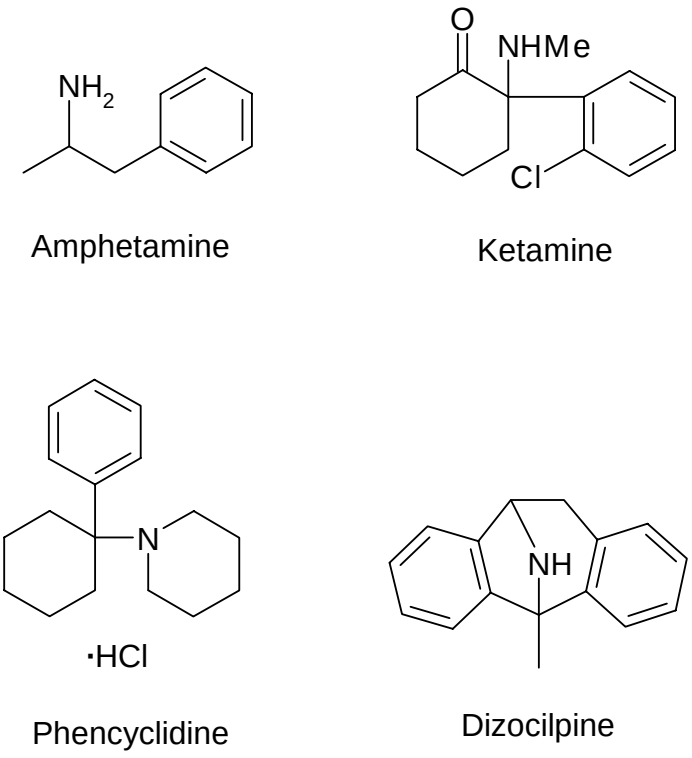

Figure 2-2: **Structural formulas of various psychotomimetic drugs.**

2.3.3 Treatment of schizophrenia

Pharmacotherapy is taken as the first and continuing measure to treat schizophrenia. Medicines are divided into two groups named typical and atypical antipsychotics. In the first group there are DA antagonists like haloperidol and the latter group contains newer drugs like clozapine. The current categorization has received criticism mostly because the name 'atypical' refers to something not used primarily while these drugs are widely used in treatment rationale (Maguire 2002).

Typical antipsychotics function by blocking D_2 receptors and help in particular with the positive symptoms producing several extrapyramidal symptoms (EPS) while atypical agents block 5-HT_2 and other receptors and do not have neurologic adverse effects in the same extend. Especially among elderly patients there is also a specific state of adverse effects called tardive dyskinesia which stands for stereotypical facial movements. This particular state can be avoided by lowering dose level gradually (Maguire 2002).

Atypical antipsychotics relieve both positive and negative symptoms as well as enchance cognition in patients. Cognitive impairment is important property in schizophrenia to treat and also psychosocial therapy is used (Breier 1999; Bellack 2001). One of the cardinal questions in the study of the mechanisms of the antipsychotics is still the lag-time of the effects as seen in figure 2-3 (Maguire 2002).

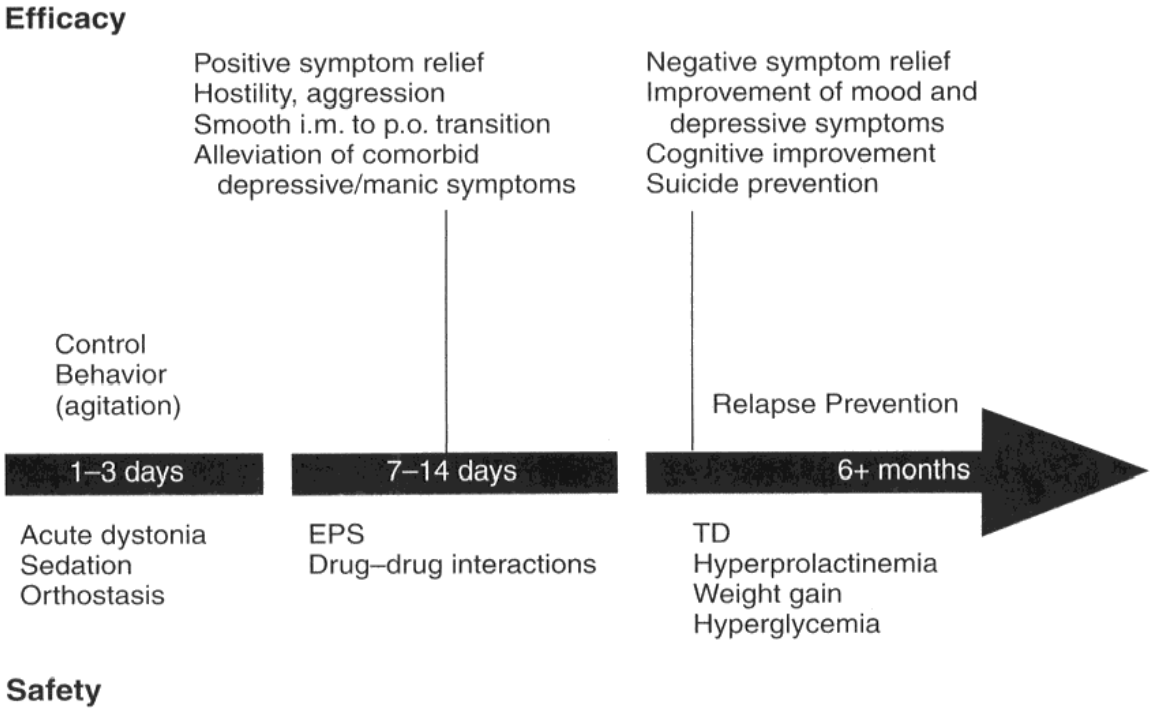

Figure 2-3: **The continuum of care for patients with schizophrenia.**
i.m.=intramuscular, p.o.=peroral administration, EPS=extrapyramidal symptoms, TD=tardive dyskinesia (Maguire 2002).

3. Prepulse inhibition phenomenon

3.1 Processing information in schizophrenia

The basic symptoms of schizophrenia suggest that there are disabilities involved with processing auditory stimuli in CNS. For example, hallucinations are mostly auditory *i.e.* patients hear something which does not exist in reality. Schizophrenia patients say that they

can not distinguish the adequate information which they receive and so they are not able to concentrate to anything (Zhang 1999). Researchers of schizophrenia have come to a conclusion this might mean deficits in sorting out stimuli *i.e.* gating of stimuli processing, which causes overflow of information finally reaching consciousness (McGhie and Chapman 1961). In addition to schizophrenia there are other disorders as well which have shown similar deficits in gating. These include, for example, obsessive compulsive disorder and Huntingtons's disease (Swerdlow *et al.* 1993; Swerdlow *et al.* 1995).

3.2 Description of prepulse inhibition

As schizophrenia is a complex disease with various characteristics and symptoms, it is unrealistic to develop just a one animal model to study the disorder. Because of that, many models have been created to express different properties of schizophrenia. Patients with schizophrenia as well as with other disorders mentioned in the previous chapter possess a deficit in sensorimotor gating which is linked to a phenomenon called prepulse inhibition (PPI) of startle. Sensorimotor gating can be measured by PPI which refers to inhibition of stimulus preceded by weak pre-stimulus. Healthy humans have got an ability to inhibit the following motor response, but schizophrenia patients show attenuated inhibition (Ludewig *et al.* 2002).

There are various possible stimuli which can be utilized to measure PPI. In the following experimental part acoustic stimulus was used and in addition to that air-puff and light are

usable. When acoustic stimulus is presented, motor response is seen in experimental animals as startling jump movement *i.e.* the whole body reacts to stimulus. In humans the startle is observed as an eye-lid movement (Zhang 1999). The acoustic startle reflex is a contraction of the skeletal musculature in response to an intense acoustic stimulus. (Ludewig *et al.* 2002). The basic measurement run is shown in figure 3-1.

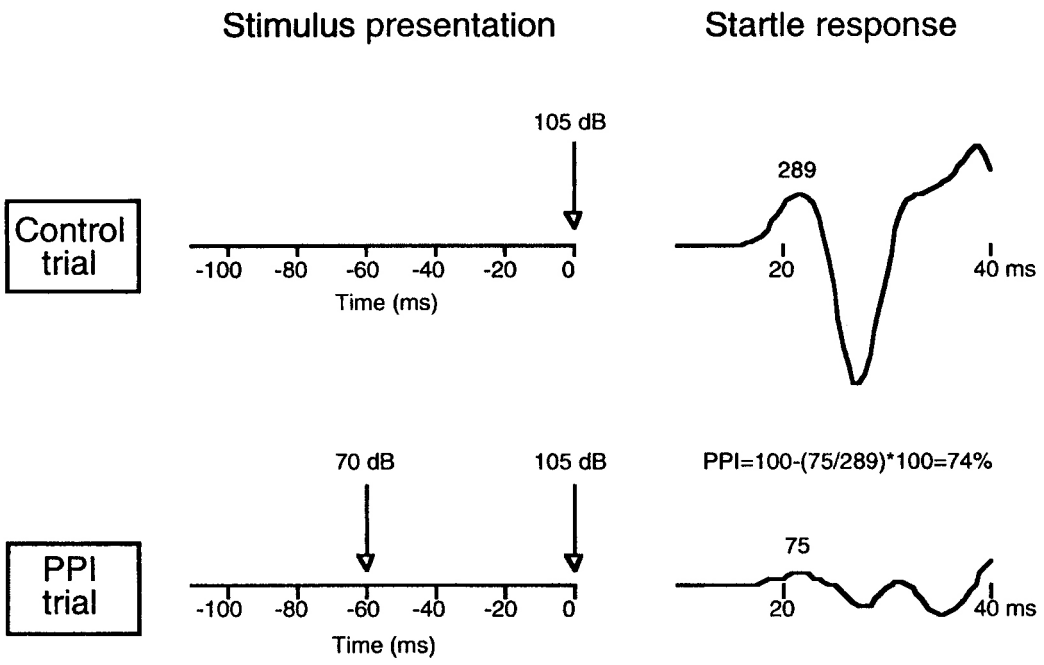

Figure 3-1: **Schematic drawing showing an actual recording of prepulse inhibition (PPI) of acoustic startle.** A noise of intensity of 105 dB is either presented alone (the control trial) or shortly after a subthreshold 70 dB prepulse (the PPI trial). The presentation of the prepulse normally results in a suppression of acoustic startle as indicated in the right part of the figure (Zhang 1999).

3.3 Pharmacological aspects

PPI of startle can be disrupted with various pharmacological agents or other manipulations and many schizophrenia models in experimental animals are also based on this observation. However, deficit in PPI is not thought to simulate schizophrenia *per se*, but more like a disturbance in sensorimotor gating (Geyer 2001). Many treatments which include psychotomimetic drugs attenuate PPI in humans as well as in animals and these alterations of PPI can be reversed by antipsychotic drugs. The modification of neuronal activity in terms of DAergic agonism no matter direct or indirect disrupts PPI. DA D_2 receptors are especially responsible for this effect (Swerdlow 1991; Zhang 1998). NA, VTA and limbic cortical regions are connected to the phenomenon as shown by microdialysis studies as well as simultaneous measurement with both microdialysis and PPI (Saunders *et al.* 1994; Zhang 2000). PCP also affects sensorimotor gating measured by PPI and in this case the NMDA receptors are blocked, but non-selectively. Dizocilpine shows selective binding on NMDA receptors (Geyer 2001). PCP affects the neurotransmission of DA in such a way that PCP alters positively DAergic firing of cells located in VTA and also increases DA release in limbic and frontal cortical areas (French and Ceci 1990; Tanii *et al.* 1990; Bakshi *et al.* 1994).

It can also be addressed that DA agonists and NMDA antagonists develop schizophrenia-like symptoms to humans. AMP and PCP representing DAergic and glutamatergic systems, respectively, pronounce psychosis, but differ in the composition of symptoms. AMP induces positive symptoms whereas PCP pronounce also negative symptoms (Javitt 1987).

3.4 Assessment of PPI as a measurable phenomenon

Animal models of schizophrenia based on PPI of startle give a possibility to evaluate sensorimotor gating deficits induced by various factors. Still, it should be assessed whether PPI of startle has got the validity that is needed to achieve the goals for both basic research and applications in the field of drug development.

PPI of startle functions non-voluntarily *i.e.* the object does not have to do anything actively to acquire the response. Phenomenon occurs in a manner of cross-species and the measurement parameters are also very similar regardless of talking about rodents, non-human primates or humans (Zhang 1999). Furthermore, a question might arise whether PPI of startle could be used in diagnostic work of schizophrenia. As explained previously, PPI deficits are not schizophrenia selective, more like occurring in various psychiatric disorders. However, in pre-clinical screening of potential antipsychotic medications the PPI models of schizophrenia are useful (Geyer 2001).

In the field of research the PPI of startle possesses good reliability in test repetition though habituation to the startling stimulus has to be controlled prior to collecting data. An issue important to notice is stress which affects the mesostriatal DA neurons involved strongly in PPI phenomenon (Doherty and Gratton 1992; Zhang *et al.* 1998). PPI models can be studied more in terms of genetically engineered animals such as knock-out animals. Furthermore, latent inhibition offers another perspective for the research of information processing of CNS (Kilts 2001).

4. Neurodevelopment and schizophrenia

Schizophrenia is seen as a brain disorder with clear genetic background. However, the entire etiology of disease can not be explained as genetic-based and there is not just one gene leading to the disorder. As shown previously, family and twin studies have brought out the environmental aspect in schizophrenia and research in the area of neurobiology has acquired more interest. Genes are still important object of studies in schizophrenia in their connection to environment. Individual's genes control the influence to environmental factors which in turn may impact one's susceptibility to several exogenous stressors (Yolken *et al.* 2000).

4.1 Course of schizophrenia

Neurodevelopmental hypothesis of schizophrenia contains postulate that neuronal development is affected by events which modify physiological course of maturation in neuronal system. These events have been suggested to be autoimmune reactions, nutritional deficiency, obstetric complications and viral exposures (Lieberman *et al.* 1997). Time-scene for the proposed factors is perinatal, which stands for period from just before to just after delivery including events during delivery. However, there is a long way to go from previously mentioned perinatal insults to the first symptoms of the actual disease which takes place most frequently at the age of puberty. The lag-time between neurodevelopmental incidents and the onset of schizophrenia can not be fully explained. There are subtle symptoms present before the onset including slight positive symptoms *i.e.* illusions and superstitiousness, mood

symptoms like anxiety, dysphoria and irritability as well as cognitive symptoms *i.e.* concentration difficulties. Anyhow, these can not be used in diagnostic sense because they are still too widely occurring in persons at this period of life who do not develop schizophrenia (Yung and McGorry 1996; Lewis and Lieberman 2000). Described premorbid events lead to schizophrenia via sensitization which eventually launches the illness and factual symptoms. Pathophysiological course of schizophrenia is shown in figure 4-1 as presented by Lewis and Lieberman.

4.2 Pathophysiology

Epidemiological studies made under last decades have prompted a greater incidence of schizophrenia in populations which have encountered previously mentioned stressors in the perinatal age of their lives. An obstetric complication, for example lack of oxygen, and a defect of nutritional quality or quantity are considered as points which raise the risk to acquire schizophrenia later. Also influenza has been taken into consideration for higher incidence of schizophrenia. However, epidemiological data should be inspected cautiously especially when compared with other similar studies as there are lots of different variations in individual studies (Weinberger 1995; Lewis and Levitt 2002).

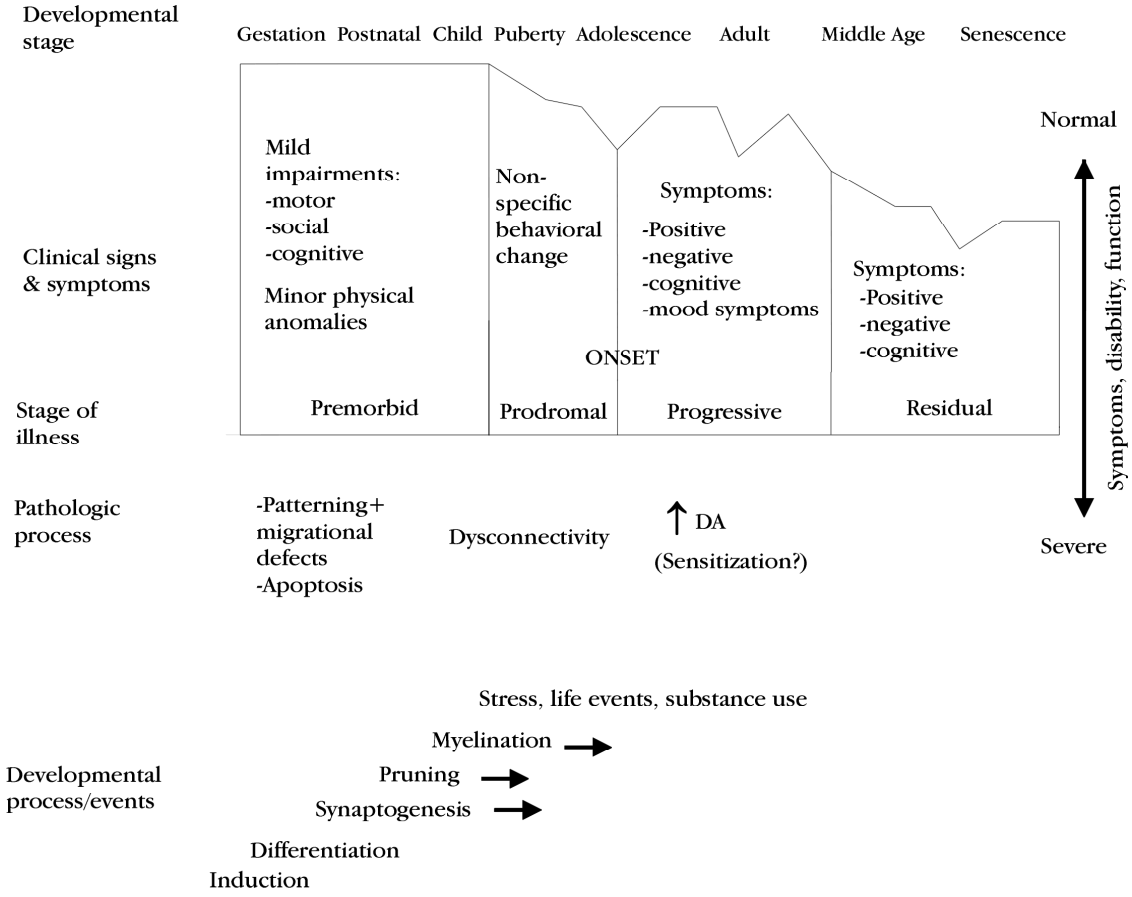

Figure 4-1: **Clinical and pathophysiological course of schizophrenia.**
The diagram attempts to integrate schematically the clinical and pathophysiological course of schizophrenia in its different clinical stages. Developmental stage shows the period of life during which the various events and insults take place. Clinical signs and symptoms refer to the mental and behavioral manifestations of the illness. Stage of illness shows the phase of the illness and the onset of the factual disease and symptoms. Pathologic and developmental process and events refer to the hypothesized pathological mechanisms which could contribute to the neurodevelopmental course of the disease and to the later events as well (Adopted and modified from Lewis and Lieberman 2000).

Brain abnormalities have been tried to find with assistance of neuroimaging device and neuroanatomical procedures. The aim has been in clarifying structural alterations in brains which would be connected to schizophrenic pathology, and in finding markers of perinatal insults described before. Neuroimaging has been carried out with computed tomography and

magnetic resonance imaging with results showing cerebral ventricular enlargement and prefrontal cortical mass reduction (Weinberger 1995; McCarley *et al.* 1999). Furthermore, these results do not correlate with the course of disorder which could mean that abnormalities are not progressive. Still, results showing ventricular enlargement have to be inspected cautiously especially in case of elderly patients, because the amount of neurones decrease naturally. Another evidence comes from histological studies which describe abnormal cytoarchitectural changes. In addition to these findings there is a consistent approval that gliosis is not encountered in schizophrenic brains. In neurodegenerative diseases glial cells express proliferation (Harrison 1999). Also animal models of schizophrenia present data that neonatal brain damage lead to behavioral effects. Summarizing all this together puts evidence on that schizophrenia is not a neurodegenerative disorder, but a neurodevelopmental one in which defects occur in early years during neuronal development (Weinberger 1995).

The final onset of the disease is preceded by changes in neuronal architecture which make brains vulnerable for the start of schizophrenic symptoms. Brains do have buffering capacity against these kinds of changes, but when this plasticity is exceeded the pathological events take place. During the age of adolescence the neuronal system is reorganized and at the same time a failure in pruning process happens which reduces redundant neuronal connections. A development of lateralization of function occurs as well as a dormant lesion in the limbic system made earlier in the neonatal period is uncovered (Feinberg 1982; Crow 1990; Weinberger 1997).

4.2.1 Sensitization

Finally, the plasticity of brains is not overwhelmed in a normal situation, but in deficiently developed brains normal life events may cause stimulation in neuronal activity and lead into sensitization. These life events include stressors as family relationship problems, going to a new school or military service or substance abuse. Neurochemical sensitization can be seen in three points of evidence: high dose of DA agonists can produce psychotic symptoms to healthy humans, low dose of agonists pronounce symptoms to schizophrenia patients and intermittent chronic use of these stimulants of abusers eventually induces the onset of psychosis (Griffith *et al.* 1968; Sato *et al.* 1992). Also stress comes into the picture as stress can sensitise the onset of symptoms as well as produce a relapse for the schizophrenic patient (Norman and Malla 1993).

5. Viruses and neurodevelopment

In epidemiological studies it has been clarified that there is connection between viral infections and schizophrenia. Babies born in winter develop more schizophrenia later in their lives than others (O'Reilly 1994). These viral insults occur during the pregnancy in terms of fetal damage or just after the delivery.

5.1 Behavioral effects of viral exposure on prepulse inhibition in animal models

As described previously, PPI of startle paradigm is widely used in the characterisation and study of sensorimotor gating. With the help of PPI, studies concerning effects of viral infection to CNS have been done. Various viruses have been used, amongst them herpes simplex virus (HSV), rat cytomegalovirus (RCMV) and influenza virus to mention a few.

HSV type 1 affects PPI in such a manner that when inoculation of virus is presented to rats neonatally in intracranial route, PPI does not develop normally but remains decreased compared to unmanipulated rats. Behavioral functions are affected selectively by the virus, as other functions than the sensorimotor gating remained normal. The other functions measured contain sensorimotor reactivity, sensorimotor response habituation, locomotor activity, rearing activity and stereotyped behavior (Engel *et al.* 2000). RCMV possesses similar effects on behavior as HSV, even when administered subcutaneously. In that study, DAergic challenge was done as well, which finally disrupted PPI of those animals exposed to RCMV (Rothschild *et al.* 1999). It has also been studied that maternal infection by influenza virus makes the offspring vulnerable to sensorimotor gating deficits like seen as attenuation on PPI (Shi *et al.* 2002). These data show evidence on neurodevelopmental hypothesis in special interest in connection between viral infections and schizophrenia development. Other viruses inspected for possible connection to schizophrenia include lymphocytic choriomeningitis virus, rubella and borna disease virus (BDV) (Monjan *et al.* 1975; Brown *et al.* 2000; Patterson 2002). The studies on neurodevelopmental model of schizophrenia in the following experimental study are based on the animal model of neonatal HSV brain infection and its effects to PPI.

5.2 Finding evidence of viral infection

Even if the epidemiological data is showing connection between viruses and schizophrenia, the molecular evidence is difficult to obtain. In viral infections, inspected either in humans or experimental animals, a trace of viral insult is complicated to find. Viral antibodies, antigens and genomes have been tried to reveal from infected patients and animals, but studies made are controversial as some of them show positive evidence on these factors while the others do not. One possibility when considering these results is that viral infections are not involved in the etiology of schizophrenia after all. Another way is that the viral traces could be present but in different brain regions than tested (Yolken and Torrey 1995). It has also been suggested that serological studies in respect to find evidence of viruses could fail because immunoglobulins are slow to develop at the neonatal period (Ahlfors *et al.* 1999). In experimental animals viral infection is hard to point out for the reason that the time-period when animals are vulnerable to infection is very short. Immunological defence mechanisms against HSV develop rapidly after birth (Engel *et al.* 2000).

5.3 Mechanisms of viral infections on neurodevelopment

Viral infections have their effects on certain brain areas, which differ depending on the virus. BDV affects hippocampus, cerebellum and neocortex and the consequences can be as dramatic as neuronal death (Weissenbock *et al.* 2000). HSV is encountered from temporal

lobe, which correlates with symptoms of human HSV encephalitis (Barnett *et al.* 1994). Neurons are lost in LCMV infection in animals after injection of virus. An acute loss is seen in cerebellum, while in hippocampus the effect is delayed (Pearce *et al.* 2000).

Viruses affect neurotransmitter systems, yet to be proven in which mechanisms. In brain monoamine systems there is some data of viral effects, but in inhibitory and excitatory systems the same has not been shown (Päivärinta *et al.* 1994; Pearce 2001).

One of the major issues in schizophrenia research is the delayed onset of the disease after neurodevelopmental insults. Viruses are known to be latent and pronounce the factual disease later. However, as discussed in previous chapters, there are no consistent results of trace of the viral infection found at the onset of the disorder (Pearce 2001). Viruses have also neurotropism characteristics, which in turn can be seen in studies as viral infections have their locations in brains in somewhat certain areas (Yolken and Torrey 1995). In animal models the viral infection is present for a short time, but still have its effects on neurodevelopment (Engel *et al.* 2000).

Finally, there are some theories proposed, which in turn might enlighten the mechanisms of the viral infections on neurodevelopment. An immune reaction of mothers could be harmful to offspring in adult life, as studied in PPI model of sensorimotor gating (Borrell *et al.* 2002). Another possibility is cytokines which are factors in the blood that mediate immune responses and are associated with many infectious and immune diseases (Yolken and Torrey 1995). Raised levels of cytokines are found in the cases of serious mental illnesses, including schizophrenia. It is also suggested that a maternal virus infection can harm the brain development of the fetus via cytokines (Patterson 2002).

6. Conclusions

The comprehension of schizophrenia has gained more enlightening knowledge from different areas of research. This has led to various theories of etiology and course of the disease, which by overlapping gather a broader outlook as schizophrenia is seen as a neurodevelopmental disorder. The genetical share in this illness is considerable, but neurobiological factors do have their crucial contribution for the actual disease to be able to develop and reach the final onset of the symptoms.

Viral infections stand for interest in neurodevelopmental studies as their capabilities to produce neuronal malfunctions in developmental period are inspected. There are several pieces of evidence suggesting viral insults affecting neuronal development. A neonatal infection in offspring is shown to affect the development of brains, but also the effects of infections to pregnant mothers can be mediated to offspring *i.e.* via cytokines. The genetical aspect has to be considered as genes in turn control responses to environmental factors *i.e.* viruses and further to potential neurodevelopmental disturbances. However, the mechanisms of viruses affecting neuronal development are not yet fully known.

Experimental part

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1. Introduction

Etiology of the one of the most difficult psychiatric disorders to treat, schizophrenia, is more understood as new approaches are taken into account. Neurodevelopment has gained more interest in recent years to get more understanding how brains are affected by various factors before the actual disease shows symptoms. After the onset of the schizophrenia the main symptoms can be summarized to abnormal sensations (hallucinations), false beliefs (delusions) and attenuation of verbal memory, decision making and attention (cognitive symptoms) (Breier 1999; Sawa and Snyder 2002). However, studies in the field of neurodevelopment are constant that the basis for schizophrenia is laid much earlier than the symptoms can be seen. Malnutrition, lack of oxygen, virus infection or just immune challenge itself are factors that contribute to the premorbid stage of the development of the disease (Borrell *et al.* 2002; Marcelis *et al.* 1998; Lewis and Liebermann 2000). These factors take place also neonatally, *i.e.* just after birth. Epidemiological studies have made it clear that there is an increased frequency between neonatal adverse events and schizophrenia. When these findings have been researched more closely with the help of histological necropsy and neuroimaging techniques, anomalously developed neuronal circuitry and decreased cortical thickness have been reported in the brain later in life (Weinberger 1995). These data connect to the involvement of the neuronal plasticity, which is attenuated later in life and these changes together launch the onset of the illness (Liebermann *et al.* 1997).

As the symptoms of schizophrenia suggest, the processing of the sensory stimuli is involved in brains. In this disorder brains fail to filter the redundant information, which comes into patient's senses disturbing or disrupting thinking and reality. With PPI phenomenon, which

refers to the inhibition of the startle reflex by a weak prepulse presented before the startling stimulus, the sensory stimulation processing and gating can be measured and further evaluated as have been proposed (Ludewig *et al.* 2002). Measurements with PPI can be carried out both on humans and experimental animals and intentional reaction is not required.

Sensorimotor gating ability is developed under neuronal development, and it is also affected by several different neurodevelopmental disturbances. Viral incidents are involved as a one factor. HSV type 1 brain infection has been shown to influence PPI of the rat (Engel *et al.* 2000) like other viruses as well (Rothchild *et al.* 1999). Neonatal HSV inoculation has been reported to lower PPI of rats later in life (Engel *et al.* 2000). Additionally, other researchers have found that viral infection, later challenged with DA agonists, is able pronounce the attenuation of the PPI (Rothchild *et al.* 1999).

The time-course of the viral infection at neonatal age in rats is short, as shown by Engel *et al.* In their experiments the viral genome was found to be difficult to find in CNS of adult rats. Human studies made earlier point out controversial results when searching viral antibodies, antigens or genome (Yolken and Torrey 1995).

Neurodevelopmental changes produce the pathophysiological basis, which is affected later in life of individual by neurochemical sensitization and leads to the symptoms of the disorder. Sensitization is described by several researchers and tested experimentally. DA agonists, both direct and indirect by mechanism of action, are competent to produce long-lasting elevation to their own effects rather than inducing tolerance (Zhang *et al.* 1998; Kalivas and Duffy 1993). Indirect DA agonist, AMP, can induce schizophrenia-like symptoms to healthy humans as

well as pronounce symptoms in schizophrenia patients. The behavioral effect of DA agonists in experimental animals tested with PPI is attenuation (Mansbach *et al.* 1988).

Sensitization prior to symptoms in schizophrenia is influenced by stressful events. The onset of schizophrenia is often related to the time of stress and in addition to that the symptoms of the schizophrenics are impaired after such experiences (Norman and Malla 1993; Liebermann 1997). These findings are connected to the role of DA by studies of stress hormones affecting DA-dependent mechanisms of sensitization (Zhang *et al.* 1998; Rivet *et al.* 1989). Corticosterone, which is a naturally occurring adrenal hormone in the rat, has got also its own effects to sensorimotor gating, as evaluated by other researchers and with different animal models (Shalev and Weiner 2001; Stevens *et al.* 2001).

Another agent known as psychotomimetic and to affect PPI is PCP. With this drug PPI can be lowered markedly. PCP is therefore used to model schizophrenia in experimental animals (Bakshi *et al.* 1994). PCP has also demonstrated to have its effects on the neurochemistry of schizophrenia by the glutamatergic mechanisms (Carlsson *et al.* 1999; Tamminga 1999).

Nitric oxide is found to be involved in the neurodevelopment of neonatal brains and suggested to be able to be blocked by nitric oxide synthase inhibitors resulting to affect in transmitter systems, particularly DA and glutamate systems (Black *et al.* 1999). Moreover, the acute effect of nitric oxide inhibition has also been found to have its effects on PCP-induced behavioral and biochemical changes in experimental animals (Klamer *et al.* 2001).

2. Materials and methods

2.1 Animals

Pregnant Sprague Dawley –rats from B&K Universal AB (Sollentuna, Sweden) were housed alone to the day when delivery (partus) took place. Rat pups were randomized to mothers next day after the birth and weaned from mothers at the age between 27-35 days. After weaning the rats were kept 3-5 per plastic cage. A 12-hour light/dark cycle was maintained and the rats were given free access to food and drinking water. Only male rats were used in the experiments. In the acute corticosterone experiment the rats (n=14) were allowed to adapt to the animal experimental facilities for seven days before carrying out the experiment. The present study was approved by the Ethics Committee for Animal Experiments, Göteborg, Sweden.

2.2 Experimental procedures

2.2.1 Prepulse inhibition -measurements

PPI of acoustic startle –measurement system (Produkt & Method, Sweden) was used throughout the study. The system consists of a plastic cage with sound-developing speakers installed to the ceiling. Rats were placed in a smaller cage, which was installed hanging from a

displacement detector. The on-going measurement was controlled and data recorded by a computer. Configurations for the measurement run and testing procedure were used as described by Engel *et al.* (2000). Tests were carried out at days 34 and 48 as control tests without any acute treatments. At day 51 in every HSV-experiment group animals were challenged by AMP.

2.2.2 Herpes simplex virus -inoculation

HSV type 1 solution from Institution of clinical microbiology (Göteborg Universitet, Sweden) was diluted with Hank's medium to concentrations of 0.25 or 0.50 plaque forming units (PFU) / 12.5 µl. Male pups were inoculated with virus in the next day after the birth intracranially to cisterna magna. While inoculation, the pups were held in isofluran anaesthesia.

2.2.3 Corticosterone administration

In the HSV-II experimental group corticosterone was administered by a pellet implanted surgically under the skin of the neck of the mothers. During the surgery rats were kept under isofluran anaesthesia. Pellet remained at place from pregnancy day 16 over partus and suckling until the pups were weaned. The HSV-III experimental group received corticosterone in

drinking water by the same time-schedule as the HSV-II group. PPI measurements were made later to the offspring of these mothers.

2.2.4 Nitric oxide synthase inhibitor administration

Nitric oxide synthase inhibitor L-NMMA was administered to the rat pups under the time period of 5-9 postnatal days. Injection was given subcutaneously daily at the same time.

2.3 Drugs

In HSV-II experimental group corticosterone pellets containing 75 mg drug were acquired from Innovative Research of America, USA. In HSV-III group corticosterone (CORT, 4-pregnene-11β,21-diol-3,20-dione, Sigma, USA) was administered in drinking water consisting 25 μg/ml drug and diluted with 0,2 % ethanol solution. Nitric oxide synthase inhibitor N^G-methyl-L-arginine (L-NMMA, 25 mg, 50 mg or 100 mg/kg, injected subcutaneously (s.c.), Sigma, USA), d-amphetamine (AMP, 1 mg/kg s.c., Apoteket Ab, Sweden), (+)-dizocilpine hydrogen maleate (0,1 mg/kg, s.c., RBI, USA) and 1-(1-phenylcyclohexyl)piperidine HCl (PCP, 2 mg/kg, s.c., RBI, USA) were dissolved in 0,9 % NaCl solution. Subcutaneously injected corticosterone used in the acute corticosterone experiment and in HSV experiments

was dissolved in 20 % 2-hydroxypropyl-β-cyclodextrin (RBI, USA), which was used as a vehicle as well.

2.4 Statistics

The data from the experiments were analyzed statistically using analysis of variance (ANOVA). Fisher's protected least-significant difference test (PLSD) was used in the acute corticosterone experiment. Values for significance were taken as $p < 0.05$.

3. Results

Corticosterone acute administration

In the acute corticosterone experiment the drug was administered in a crossover design in a manner that each animal had all dose levels of an experiment (5 mg, 10 mg and 20 mg/kg). Pulse alone trials showed no significant differences between vehicle treatment and corticosterone treatment at any dose used (Appendix 2). The same was observed in the habituation trials. In the PPI trials (Figure 3-1), however, a significant increase was observed between vehicle treatment and all treatment doses ($P<0.05$).

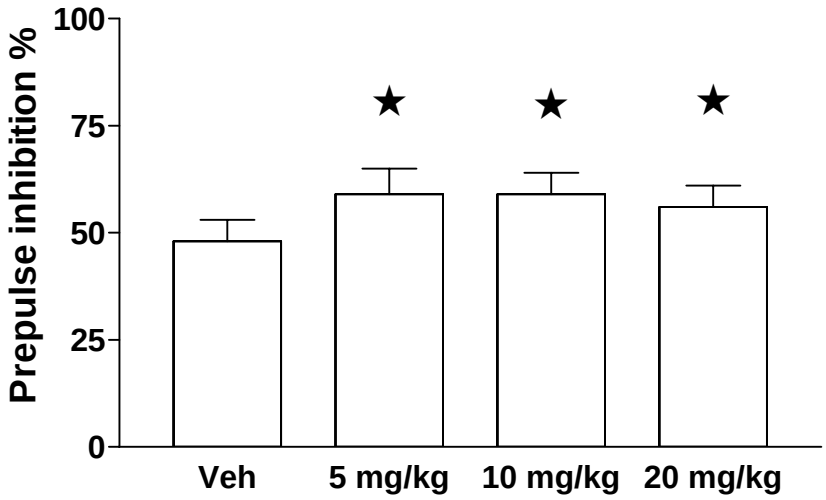

Figure 3-1. **Acute effect of CORT at various dose levels (5 mg/kg, 10 mg/kg and 20 mg/kg, s.c.) on PPI of acoustic startle.** The results are represented as means ± SEM (n=15), * P<0.05

Experiment HSV-I (HSV 0.25 PFU, L-NMMA 100 mg/kg, Fe2+ 10 mg/kg)

Neonatally HSV -infected (0,25 PFU) rats showed increase in pulse alone trials at AMP challenge (1mg/kg) at postnatal day 52. PPI observed to be disrupted significantly at the same test. Nitric oxide synthase inhibitor L-NMMA (100 mg/kg) given daily during postnatal days 5-9 caused a decrease in pulse alone startle response observed throughout the study. Similarly, a lowering effect of L-NMMA on weight was seen. At postnatal day 77 test made with PCP (2 mg/kg) did not cause significant attenuation of PPI at any of the neonatal treatments and the same was noticed with AMP both at day 73 (1 mg/kg) and at day 89 (0,5 mg/kg).

Experiment HSV-II (HSV 0.5 PFU, L-NMMA 50 mg/kg, CORT 75 mg/21 days)

L-NMMA (50 mg/kg) did not have any effect on measured parameters over the whole test period. On this test made at day 51, also AMP (1 mg/kg) did not show effects on PPI or pulse alone startle response. HSV (0,5 PFU) treated animals had slight but non-significant effects on measured parameters in control tests (days 34, 48, and 65) as well as AMP challenge and CORT (2,5 mg/kg) administration at day 69 (Figure 3-2). The rats, whose mothers had received CORT by pellet (3,57 mg/24 h), also did not show any significant changes in parameters. L-NMMA treated animals showed increased PPI on CORT administration (2,5 mg/kg) at day 69 compared to control test made at day 65 ($P<0.05$).

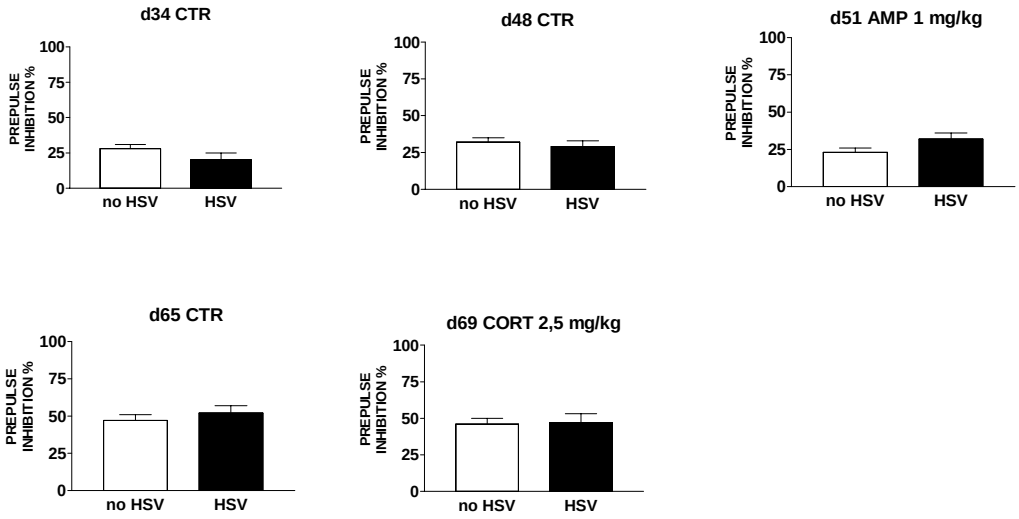

Figure 3-2. **Experimental group HSV-II: The effect of neonatal inoculation of HSV type 1 (0.5 PFU) and later challenge treatments (AMP 1 mg/kg s.c.; CORT 2,5 mg/kg s.c.) on PPI of acoustic startle.** The dose levels of later challenge treatments and respective ages of rats are shown in figure. The results are represented as means ± SEM (n=18-26). CTR = control recording.

Experiment HSV-III (HSV 0.25 PFU, L-NMMA 25 mg/kg, CORT 2.5 mg/100 ml/21 days)

HSV (0.25 PFU) treated animals did not show any effects on PPI, either in CTR-tests or later challenge tests (Figure 3-3). Perinatally administered CORT (1,5 mg/24 h) to mothers in drinking water increased weight (Appendix 1) significantly at days 34, 48 and 51 in rat pups ($P<0.05$) and decreased PPI (Figure 3-4) at day 48 ($P<0.01$). L-NMMA (25 mg/kg) caused decreased PPI at day 34 control test ($P<0.01$). Later on at day 65 test made with dizocilpine at the dose level 0,1 mg/kg did not show any effects between different neonatally treated groups.

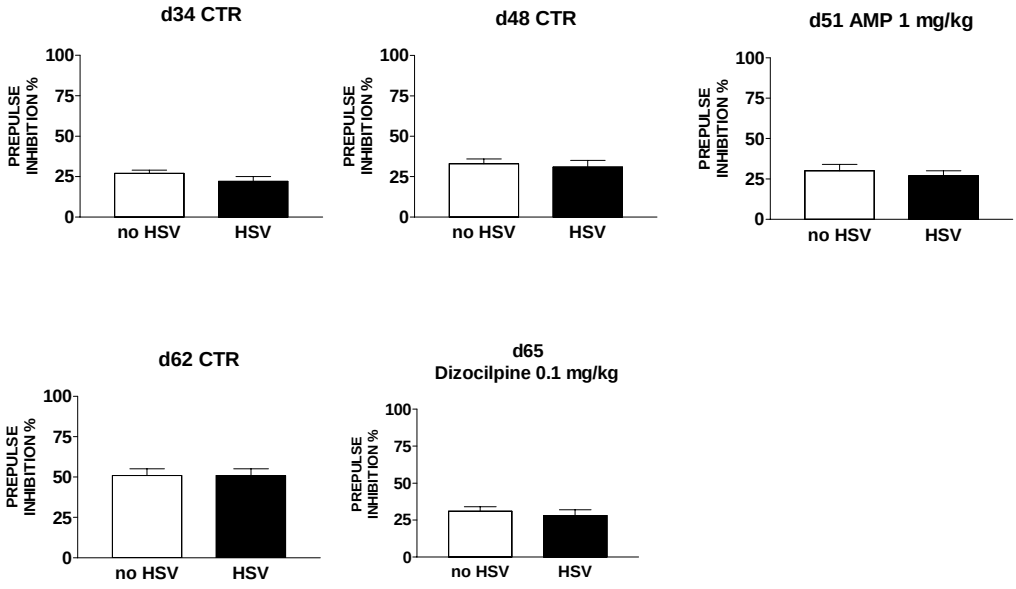

Figure 3-3. **Experimental group HSV-III: The effect of neonatal inoculation of HSV type 1 (0.25 PFU) and later challenge treatments (AMP 1 mg/kg s.c.; dizocilpine 0,1 mg/kg s.c.) on PPI of acoustic startle.** The dose levels of later challenge treatments and respective ages of rats are shown in figure. The results are represented as means ± SEM (n=26-28).

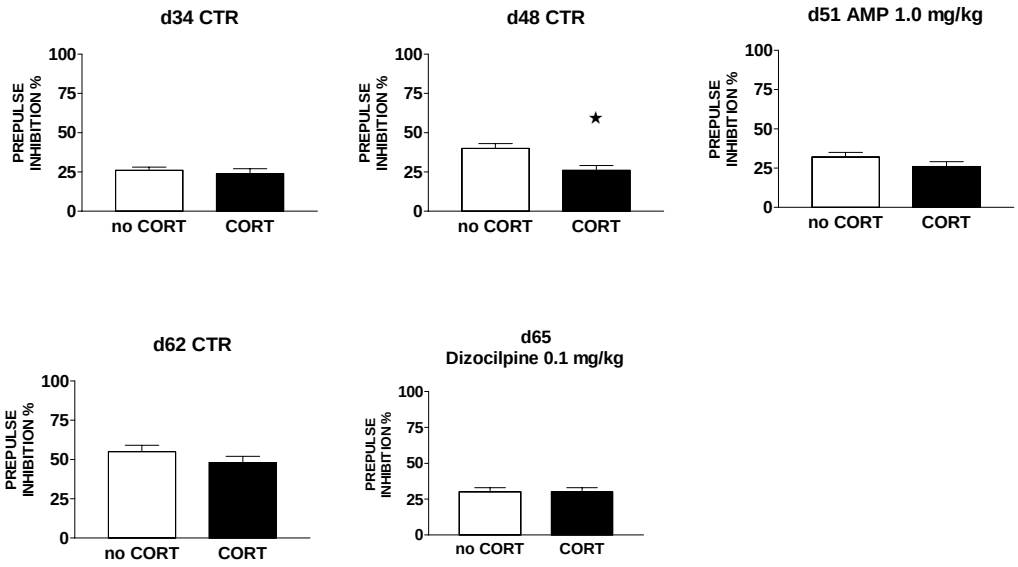

Figure 3-4. **Experimental group HSV-III: The effect of perinatal administration of CORT (1,5 mg/24 h) to mothers and later challenge treatments (AMP 1 mg/kg s.c.; dizocilpine 0,1 mg/kg s.c.) on PPI of acoustic startle.** CORT was administered to mothers by drinking water beginning 7 days before partus until weaning. The dose levels of later challenge treatments and respective ages of rats are shown in figure. The results are represented as means ± SEM (n=26-28), * $P<0.05$.

4. Discussion

The present study was made to find out consequenses of connections between perinatal CNS disturbances and their effects to sensorimotor gating in animals. The modelling for different factors affecting the development of CNS were made to the rat by neonatal HSV infection and by perinatal corticosterone administration to rat mothers. These effects were both counteracted by neonatal administration of nitric oxide synthase inhibitor L-NMMA and later challenged with DAergic and glutamatergic substances as well as with corticosterone.

Totally three different experimental studies were carried out. The first of them showed effects to sensorimotor gating after HSV-treatment made at neonatal period. These lowering effects on PPI of acoustic startle were observed at AMP (1mg/kg) challenge at day 52, which shows the sensitising behavioral effect to DA agonist and could be compared to the earlier studies of sensitization (Liebermann 1997). At the same test the sensorimotor reactivity as measured by extent of acoustic startle response (ASR) was improved. AMP given alone has not been shown to affect this response in previous studies (Zhang *et al.* 2000). Habituation to the sensorimotor response was not affected and the similar trend was seen throughout the study. However, in the two following studies similar significant effects to sensorimotor gating could not be seen. In the second experimental group, HSV-treatment caused a slight effect to acoustic startle and PPI, but on the latter one the effect was rather improved than attenuated. At the last of the studies HSV-effects were not observed, which gives suspicion to the overall results. The inoculated HSV-concentration differed between the experimental groups, but in the first and the third ones it was kept the same at 0.5 PFU. In the search for possible reasons to the variation on these results, the biological characteristics of HSV have to be taken into account. There might be differences between animals by the infection, especially the time-period of the infection has to be considered to be very narrow (Engel *et al.* 2000).

In HSV treated animals given either PCP or dizocilpine, both NMDA-receptor antagonists, no disruption on PPI was observed similarly to those not inoculated by virus. These results compared with AMP challenge test may presume that the glutamatergic system is not affected in the same manner as the DAergic system after neonatal virus infection.

Sensorimotor reactivity was lowered persistently with the neonatal treatment of L-NMMA at the dose level of 100 mg/kg. This observation may have been an outcome of the lowered weight of the animals, which also was persistent compared to the animals which were not given nitric oxide synthase inhibitor. Similar effect on weight was not seen with lower dose levels, but effects to sensorimotor gating disappeared as well. An exception to this was L-NMMA at the dose level of 25 mg/kg which showed significantly lowered PPI on control test at day 34. However, this effect disappeared in the later tests and therefore it may have been a result of slow maturation. When compared to follow-up study made by Engel *et al.*, PPI develops fastest just at this age of the rat.

Administration of corticosterone to the mothers of the rats occurred in two different ways, by an implanted pellet or by drinking water. This was made to model stress which can affect the normal neuronal development of the pups. At the HSV-II group could not be seen any significant effects of CORT, as a matter of fact some mothers got rash and some of them showed disturbed behavior during suckling. However, considering the HSV-III group, administered in the drinking water, CORT caused significant and persistent improvement on weight over age from postnatal day 34 to day 51. Effects to sensorimotor gating were seen as lowered PPI at day 48 control test. AMP (1 mg/kg) treatment did not disrupt PPI as observed between CORT treated and non-CORT treated animals. Administration by a pellet caused less effects to measured parameters even though it released CORT over twice (3.57 mg/24 h) as much as administration by drinking water (1.5 mg/24 h). The probable reason to this may be the feedback loop of cortical hormones as a pellet releases CORT continuously (3.57 mg/24 h) and causes negative effect to the loop. Rats drink intermittently and therefore the administration by the water increases systemic CORT level at a pulsatile manner. One

possibility to avoid problems at pellettal administration could have been to carry out adrenalectomy of the animals as was done by Stevens *et al.* (2001).

CORT (2,5 mg/kg) was given also in terms of acute administration after different neonatal pre-treatments. Slight but non-significant effect of decreased ASR was observed without any effects on PPI. The acute experiment made to drug naïve animals in a dose-response manner showed significant improvement on PPI but no effects to ASR. Controversial results obtained in these studies do not acquire more enlightening clarification from literature, just chronic CORT administration have been tested for sensorimotor gating before.

In summary, the studies carried out made it clear that disturbances caused by various treatments under perinatal period of life can affect neuronal development in terms of disorders later in puberty and adulthood. However, the measured parameters showed most of the alterations at pubertal time period and disappeared or diminished as animals grew up. This may be an outcome of the compensatory capacity of the CNS to alleviate such changes. On the other hand, previous studies have shown that the effect of HSV infection can persist to the adulthood (Engel *et al.* 2000). Neurodevelopmental changes after neonatal HSV infection are to be studied further before the role of the viral infection can be related stronger to mechanisms of CNS developmental disturbances.

Acknowledgements

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Appendix 1.

The effect of perinatal administration of corticosterone and later challenge treatments on weight of the animals

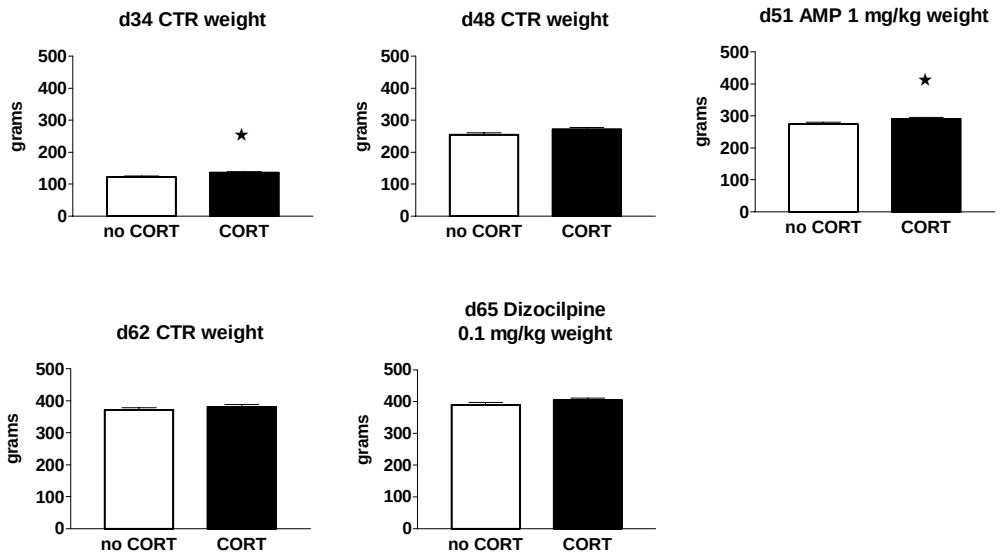

Figure 1-1. **Experimental group HSV-III: The effect of perinatal administration of CORT (1,5 mg/24 h) to mothers and later challenge treatments (AMP 1 mg/kg s.c.; dizocilpine 0,1 mg/kg s.c.) on weight of the animals.** CORT was administered to mothers by drinking water beginning 7 days before partus until weaning. The dose levels of later challenge treatments and respective ages of rats are shown in figure. The results are represented as means ± SEM (n=26-28).

Appendix 2.

The effect of acute corticosterone on acoustic startle response

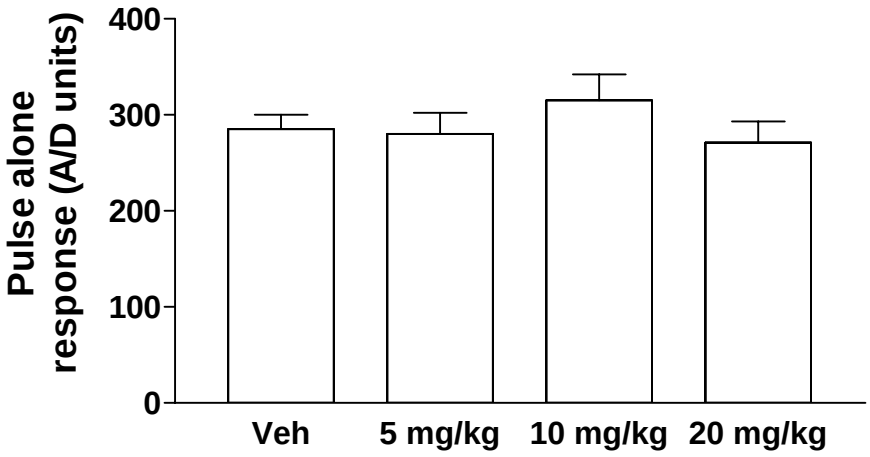

Figure 2-1. **Acute effect of CORT at various dose levels (5 mg/kg, 10 mg/kg and 20 mg/kg, s.c.) on acoustic startle response.** The results are represented as means ± SEM (n=15)