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# Finding an animal model of schizophrenia: Combining induction strategies to create new research opportunities.

**Bachelor thesis**

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# Content

## Abstract

For many years, animal models are of great importance for biological and pharmacological research. They help in revealing novel information about disease and provide assistance in developing treatment strategies. However, treatment of the neurodevelopmental disorder schizophrenia is still insufficient, mainly because the underlying biology of this devastating disorder is largely unknown. Because of the complexity of onset and development of schizophrenia, creating an animal model possessing all the characteristics is extremely hard, or even impossible. In this thesis the criteria of an animal of schizophrenia and the different methods of creating such an animal model are discussed. It is important to generate an animal model that matches the frequently proposed two (third) hit hypothesis of schizophrenia, stating that a series of stressfull events to a genetically vulnerable individual leads to the development and onset of schizophrenia. To create an animal model that mimics this neuropathology best, combinations of induction strategies should be tested for face, construct and predictive validity, ultimately resulting in an animal model possessing as many of the characteristics of schizophrenia as possible. Recent results reveal that inbred Roman High Avoidance and Roman Low Avoidance rats possess promising characteristics and should therefor be further tested for suitability in schizophrenic research. Combined with a proper feedback cycle from clinical findings this can lead to a valuable animal model generating better understanding and development of preventative strategies for schizophrenia.

## Introduction

Animal models are widely used to study aspects of behaviour and physiology that are not accepted to be studied in humans. This general acceptance of animal studies resulted in many insights in both animal and human physiology and, most important, pathology over the past decades. Rodent studies in particular provided insight in several aspects of neurobiology and neurodegenerative diseases, such as Alzheimers disease (Lannfelt et al., 1993) and Parkinsons disease (Reader & Dewar, 1999).

In this thesis the use of animal models in relation to the neuropsychiatric development disorder schizophrenia is discussed. Over the past decade many studies have been done in order to understand schizophrenia and to improve medication, therapy and thereby quality of life. However, since schizophrenia is a very complex and multifactorial disorder, this research is an ongoing process and many aspects of –the development of- schizophrenia are still poorly understood. In order to understand the seriousness of schizophrenia and the necessity of improved insight in the underlying mechanisms, the disorder will first be discussed shortly.

The name schizophrenia is derived from the early observation that it is characterised by "the splitting or disconnection of the psychic functions" (Greek: *skizhein:* "to split" and

*phrenos*: "mind"). However, patients suffering from schizophrenia do not always suffer from the well-known "split personality". Instead, symptoms that are often associated with schizophrenia are the lacking of insight, hallucinations like "hearing voices", delusions such as the false feeling of being part of a conspiracy and thought disorders such as speech problems and randomized movement (Picchioni & Murray, 2007). Having these most common symptoms in mind, it is easy to understand that the output of the disease is extremely hard to measure in an animal model. Indeed, it is impossible to score the presence of hallucinations and delusions in animals. Studying schizophrenia in animals is therefore always restricted to physiological and behavioural parameters.

Human post mortem studies revealed neuropathological symptoms of schizophrenia that can be useful as possible fields of interest to focus on in animal studies. Several studies show decreased quality and quantity of neurons, synapses and dendrites in the schizophrenic brain (Iritani, 2013). Molecular studies also revealed many genes that are affected in schizophrenic patients. Many of these are associated with the development of neurons, axons, synapses, glial cells and neurotransmitters in the brain. For example, the DISC-1 (Disrupted-In-Schizophrenia-1) is currently the most credible candidate gene for schizophrenia and is involved in migration and elongation of neurons in the developmental period (Kamiya et al., 2005).

However, these findings are still based on post mortem human studies. Since schizophrenia is a neurodevelopmental disorder, insight in the early stages of this disease is essential for developing therapies that can be useful in early-stage treatment. Since early-stage studies in humans are ethically unfeasible, animal studies can be very helpful. Thus, a paradox in studying schizophrenia exists between the impossibility to score schizophrenia in animals based on the symptoms mentioned earlier on the one hand and the difficulties to study human schizophrenia in early stages on the other.

Though animal models for a quintessential disease such as schizophrenia are very hard, and perhaps even impossible (C. M. Powell & Miyakawa, 2006) to create, many animal models have been developed in order to investigate early stage development of schizophrenia (Young, Zhou, & Geyer, 2010). However, animal studies most often only study one or few aspects of the disorder and are therefore limited. All animal models that have been used can be divided into four different induction categories: developmental, drug-induced, lesion, or genetic manipulation(Jones, Watson, & Fone, 2011; 2012). These four categories will be described more extensively later in this thesis.

Despite many studies on this disorder that was formerly known as "the graveyard of neuropathologists" (G. W. Roberts & Bruton, 1990), treatment is very difficult. Many antipsychotic drugs administered to patients result in side effects considered worse than the disorder itself: urging abortion of treatment and thereby a comeback of symptoms. Side effects (listed in Appendix 1) such as

hypotension on the long term increase mortality and morbidity, whereas extrapyramidal effects decrease the quality of life. Therefore, better understanding of schizophrenia is essential in order to improve lifespan and quality of life for patients suffering from this devastating disease.

In order to do so, animal studies are inevitable and will allow researchers to study fundamental underlying mechanisms that will help in improvement of medication. However, as mentioned before, animal models are very difficult to create and bring a lot of limitations. Thus, improving these animal models is crucial in the future of schizophrenic research and neuropathological research in general.

Recently, many new insights in animal studies support the idea that animal models should be fit for studying the disorder of interest. Individual variation is widespread among humans and animals and can be extremely valuable in research on a particular subject or disease (Koolhaas, de Boer, Coppens, & Buwalda, 2010). Indeed, pathology is an extreme phenotype and research on pathology should therefore be done in matched animal models in order to simulate the effects of potential medication on this phenotype.

In this thesis the current situation on animal models for schizophrenia will be discussed. By explaining results from studies in other animal models and combining them with the results of research we did last year, an

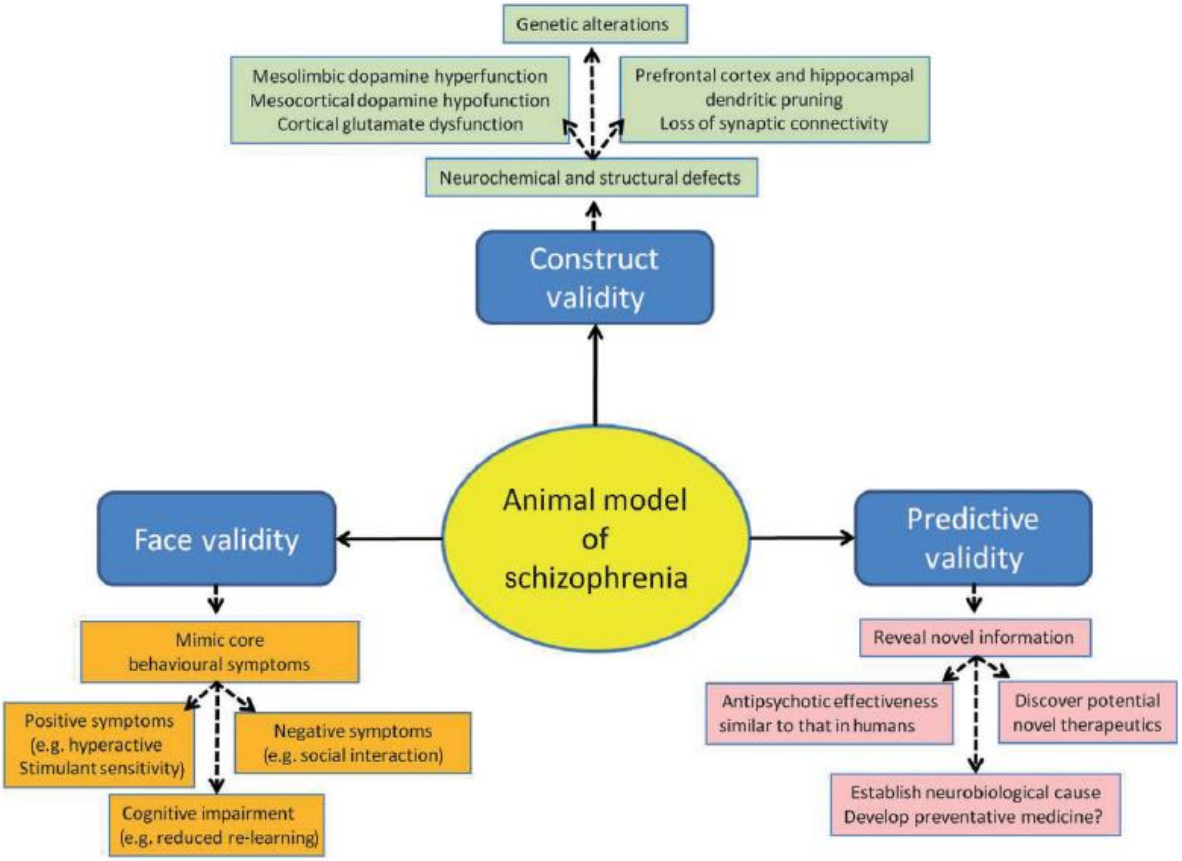



**Figure 1** Schematic diagram of the key behavioural, neurochemical and structural changes expected be present and to have translational relevance to the three core symptom domains of schizophrenia in an animal model of the disorder. *(Jones et al. 2011;2012)*

answer to the research question will be provided: what animal model would be the most suited for studying schizophrenia?

## Requirements of an animal model of schizophrenia

Finding the most suited animal model for schizophrenia initially starts with knowledge of the existing models. There are some criteria that an animal model should meet. An ideal animal model should have all three aspects shown in figure 1. First, it has to mimic the positive, negative and cognitive behavioural symptoms that are typified in schizophrenia in humans. Second, it has to possess the neurochemical and structural defects. And finally, an animal model should have great predictive value in testing new drugs or revealing underlying mechanisms and potential novel therapies. Before discussing the four induction categories used in animal models it is important to clarify the validities shown in figure 1 first.

### *Face validity*

The face validity requires similar symptom manifestation to the clinical condition (McGonigle, 2013). In schizophrenia, three types of symptoms are classified: positive symptoms, negative symptoms and cognitive symptoms. Some of the symptoms and their corresponding cognitive systems are listed in table 1.

*Positive symptoms*

Positive symptoms are symptoms (or signs) from a lower level of evolution that would break through higher levels of evolution due to a loss of higher level brain regions (Andreasen, 1995). Symptoms include hallucinations, delusions, disorganised speech and disorganised behaviour. It is questionable whether animals suffer from hallucinations and delusions. And if so, we have no means in measuring them. Moreover, disorganised language is even harder to measure in animals.

Therefore, measuring positive symptoms of schizophrenia in animals is restricted to measuring surrogate behaviour. A response to an antipsychotic drug by measuring locomotor activity provides information about a possible parallel between a schizophrenic patient and an animal model and could therefore be used for finding animal models for schizophrenia (C. M. Powell & Miyakawa, 2006).

Another possibility for measuring positive symptoms is using the fact that dopamine and dopamine receptor expression is upregulated in the schizophrenic brain (Zakzanis & Hansen, 1998). Since the dopaminergic system influences psychomotor activity, measuring psychomotor agitation in an open field test as a readout of alterated dopaminergic expression could be assessed (G.F. Koob, 2012).

*Negative symptoms*

Negative symptoms are symptoms that are expressed as a result of loss of function pathology in the brain. It is important to know that symptoms in a cluster are correlated, but

positive symptoms are *not* correlated with negative symptoms (Andreasen & Olsen, 1982). This indicates that different neuropathology pathways all lead to the disorder "schizophrenia", but are reflected differently in patients.

Negative symptoms include alogia, apathy, affective blunting, anhedonia and avolition (Andreasen, 1995)

Again, many of the symptoms such as alogia, apathy and affective blunting are virtually impossible to model in laboratory animals. Therefore, main focus of modelling the negative symptoms is on measuring anhedonia and social withdrawal (Ellenbroek & Cools, 2000).

Anhedonia can be measured by tests that include activation of reward systems in the brain. Self-stimulation is a widely used method in which animals suffering from anhedonia would experience less reward from the stimulation and thus would increase the stimulation threshold or reduce the breaking point in a progressive ratio schedule of self-stimulation (Ellenbroek & Cools, 2000).

Social withdrawal is a paradigm not so difficult to study in animals. A resident intruder test in which the receiving animal is one of low aggression can be used for studying several social behaviours. Animals that express lower levels of social interaction are therefore regarded as socially withdrawn animals (G.F. Koob, 2012).

However, since effective treatment of the negative symptoms by antipsychotic drugs is lacking, proper testing the validity of anhedonia and social withdrawal as useful paradigms in studying schizophrenia has yet to be done.

*Cognitive symptoms*

The last group of symptoms described in schizophrenia is the group of cognitive symptoms as a result of the disorder. Tasks of attention, tasks that include executive functions or declarative memory, and working memory are often disrupted in schizophrenic patients (van Os & Kapur, 2009). These symptoms are perhaps the easiest to study in animals because of the fact that they are not exclusively human.

The failure of correct preattentive information processing correlates with the oversensitivity to sensory stimulation and cognitive fragmentation in schizophrenic patients (Braff, 1990). This can be studied in animals using prepulse inhibition (PPI) of the startle reflex as a paradigm behaviour (Swerdlow & Geyer, 1998).

In a typical PPI test

| Cognitive system or subsystem | Schizophrenic symptom |
|---|---|
| | *Positive* |
| Perception | Hallucinations |
| Inferential thinking | Delusions |
| Language | Disorganised speech/formal thought disorder |
| Behavioural monitoring | Disorganised/bizarre/catatonic behaviour |
| | *Negative* |
| Conceptual fluency | Alogia |
| Emotional expression | Affective blunting |
| Experiencing pleasure | Anhedonia |
| Volition | Avolition |

**Table 1.** Relation between cognitive systems and schizophrenic symptoms *(Andreasen, 1995)*

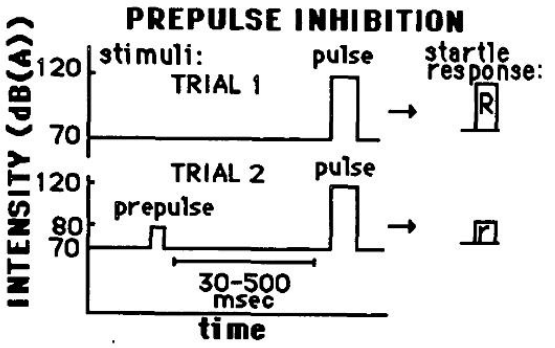



**Figure 2** Comparison of a startle reflex response as a result of a pulse alone (TRIAL 1) and a startle reflex after a prepulse (TRIAL 2). The degree to which the prepulse inhibits the response provides a measure of sensorimotor gating *(Swerdlow & Geyer, 1998)*

an animal is exposed to a soft auditory stimulus before being exposed to a harder stimulus (figure 2). By measuring pressure on the platform the animal is sitting on, the response to the second stimulus can be measured. Animals that suffer from deficit preattentive information processing will respond fiercer to the second stimulus, providing information about stimulus processing and thereby suitability of being a schizophrenic animal model.

Another behavioural paradigm to test cognitive deficits is the latent inhibition (LI) test. Latent inhibiton is a well conserved mechanism observed in many animals that prevents associative conditioning when combined with a familiar stimulus compared to conditioning with a new stimulus. In other words, when an animal is already familiar with an auditory stimulus, it is harder to condition it with a reward compared to an animal that is not yet familiar with the same auditory stimulus. This mechanism is thought to prevent an excess of information in animals.

This test is relevant in studying schizophrenia because of the observation that schizophrenics are unable to ignore irrelevant information (Maher, 1983). Therefore, this test has already been used in studying schizophrenic humans (Lubow & Gewirtz, 1995) and can be a valuable paradigm behaviour in studying schizophrenia in an animal model.

In addition to the Prepulse Inhibition and the Latent Inhibition tests, many other cognitive behavioural tests can attribute in studying schizophrenia in animals. Working memory (radial arm maze), cognitive flexibility (Morris water maze), sustained attention (5-choice serial reaction time test (Bushnell PJ, 2009)) and inhibitory response control (object retrieval test (A. C. Roberts & Wallis, 2000)) are also associated with alterations in the prefrontal cortex and schizophrenia (G.F. Koob, 2012; Holmes & Wellman, 2009). In this thesis no further details about these tests will be discu, but it proves that many options to test the cognitive symptoms are available.

### *Predictive validity*

Next to the face validity a second validity, which requires responsiveness to clinically effective therapeutic agents, is the predictive validity (McGonigle, 2013). It is important for an animal model to be able to predict certain outcomes of human research. This provides direction to many human studies and diminishes the number of potential drugs to be tested in humans.

Therefore, it is of great importance that human studies are carefully devised and analysed afterwards. When the outcome of a

human study significantly differs from the prediction based on an animal study, this inevitably means a restriction of the animal model being appropriate for studying schizophrenia. In that case the effects of that certain drug should be re-examined in other (more suited) animal models. Moreover, potential drugs dismissed from clinical follow up based on the initial animal model should be reconsidered as being potential therapeutic agents and tested again in the new animal model.

This feedback loop will exclude animal models that are not suited for schizophrenic studies and select models that are predictive. Combined with (genetic) alterations of the existing and high predictive validity animal models this will lead to animal models that are of great value for neuropathological research.

Ultimately, this process will not only reveal novel therapeutics, but simultaneously creates an animal model that offers more insight in the underlying mechanisms and might help in establishing the neurobiological cause of schizophrenia. This, in turn, helps to develop preventative medicines and leads to delay of onset and improved quality of life of schizoprenics (Jones et al., 2011; 2012).

### *Construct validity*

The third validity is the construct validity that requires similar underlying biology of the animal model compared to the human situation (McGonigle, 2013). In order to compare underlying biology, it is important to describe the neuropathology of schizophrenia first.

Over the past years many risk genes of schizophrenia have been proposed, reviewed in 2005 by (Harrison & Weinberger, 2005). As shown in Table 2, genes differ in strength of evidence in many domains, making it impossible to designate "the schizophrenic gene". This results in many points of view on the role of genes in schizophrenia.

Clearly, genes are involved in the neuropathology of schizophrenia. This idea is supported by epidemiological studies showing a 48% risk of developing schizophrenia when genetic overlap with a schizophrenic patient is 100%, compared to a global lifetime prevalence of 1% (Tsuang, 2000).

Further evidence of a genetic base comes from the observation that many of the genes associated with schizophrenia are involved in synaptic plasticity and development and stabilization of cortical microcircuitry. These findings might in part explain the morphological correlates found in schizophrenia, ranging from a slight reduction in brain size to localized alterations in the morphology and molecular composition of specific neuronal, synaptic, and glial populations in the hippocampus, dorsolateral prefrontal cortex, and dorsal thalamus (Harrison & Weinberger, 2005).

However, the exact mechanisms by which genetic mutations of these genes lead to the symptoms remain a matter of debate. It has been suggested that schizophrenia is a homogenous pleiotropic disorder, meaning that multiple genes all contribute to the disorder partly with different contribution of different alleles (Tsuang, 2000).

However, this "common disease – common alleles" model is countered by a new hypothesis which suggests the idea of a "common disease – rare alleles" disorder. In other words, according to this hypothesis, schizophrenia is highly heterogeneous genetically and mutations of the alleles are individually rare and highly penetrant.

This idea is supported by the lack of evidence for global homogenous mutations. Instead, multiple studies show different outcomes for two promising candidate genes of schizophrenia, dysbindin and DISC-1. For instance, variants of dysbindin are associated with an increased disease risk in some studies and a decreased risk in others (Owen, Williams, & O'Donovan, 2003; 2004).

Also, so far no common polymorphisms of DISC-1 have been found across populations. In Europe and North-America, haplotypes of DISC-1 have been associated with schizophrenia, but in Japan and Scotland this was not the case. This absence of strong evidence for common alleles supports the idea that rare mutations in small populations or even families cause the disease, instead of common mutations worldwide (McClellan, Susser, & King, 2007).

This idea of course complicates the development of an animal model possessing high construct validity with regard to genetic alterations. However, the high, but definitely not maximal rate of inheritance indicates that much more aspects contribute to the biological mechanisms causing the symptoms of schizophrenia.

**Table 2** Schizophrenia susceptibility genes and the strength of evidence in four domains (Harrison & Weinberger, 2005)

| *Gene*[a] | *Locus* | *Strength of evidence (0 to + + + + +) for* | | | |
|---|---|---|---|---|---|
| | | *Association with schizophrenia*[b] | *Linkage to gene locus*[c] | *Biological plausibility*[d] | *Altered expression in schizophrenia*[e] |
| COMT | 22q11 | + + + + | + + + + | + + + + | Yes, + |
| DTNBP1 | 6p22 | + + + + + | + + + + | + + | Yes, + + |
| NRG1 | 8p12–21 | + + + + + | + + + + | + + + | Yes, + |
| RGS4 | 1q21–22 | + + + | + + + | + + + | Yes, + + |
| GRM3 | 7q21–22 | + + + | + | + + + + | No, + + |
| DISC1 | 1q42 | + + + | + + | + + | Not known |
| G72 | 13q32–34 | + + + | + + | + + | Not known |
| DAAO | 12q24 | + + | + | + + + + | Not known |
| PPP3CC | 8p21 | + | + + + + | + + + + | Yes, + |
| CHRNA7 | 15q13–14 | + | + + | + + + | Yes, + + + |
| PRODH2 | 22q11 | + | + + + + | + + | No, + |
| Akt1 | 14q22–32 | + | + | + + | Yes, + + |

The ratings are of course subjective and transient.
[a]Gene names, in the order they are discussed in the text.
[b]Based on sample sizes and numbers of replications, not the magnitude of the relative risk. + + + = at least three positive independent studies.
[c]Based on the meta-analyses[164,165] and individual studies.
[d]Based on information regarding expression and function *in vivo* or *in vitro*.
[e]Abundance of mRNA or protein, or the relative expression of isoforms or alleles.

One of the possible explanations for this lies in the field of epigenetics. Epigenetics are believed to play a major role in the onset of genes via DNA methylation or histone modification, hereby regulating transcription of genes and ultimately synthesis of proteins. It has been shown that in the prefrontal cortex of schizophrenics, a region often affected in these patients, abnormal DNA or histone methylation of specific genes causing alterations in RNA expression is observed (Akbarian, 2010).

Both genetic and epigenetic alterations are the in some way underlying cause of the neurochemical and structural defects observed in schizophrenia. Therefore, it is of great importance that these factors of the construct validity are taken into account when searching for a valid animal model.

## Induction categories

As mentioned before, there are four distinct induction categories when creating an animal model for schizophrenia: neurodevelopmental, drug-induced, lesion or genetic manipulation (Jones et al., 2011; 2012). In this chapter all four categories and some examples considering the criteria mentioned in the previous section will be described.

### *Neurodevelopmental models*

It has been shown in many human epidemiological studies that exposure of an individual during gestation or in the perinatal period to environmental insults increases the risk of developing schizophrenia. Examples of these insults are malnutrition, infection or immune activation and obstetric complications (such as hypoxia) during birth (Lewis & Levitt, 2002).

Multiple animal models have been developed based on this principle making use of the fact that the precise nature of the adverse is not important per se, the time that it occurs however is. Manipulation of environment or drug administration during this sensitive perinatal period create animal models with irreversible disruption of CNS development.

Examples of these manipulations include disruption of neurogenesis during a critical gestational period, neonatal ventral hippocampus lesions, post-weaning social isolation and perinatal or maternal immune activation of rodents (Jones et al., 2011; 2012).

An example of such an animal model is the gestational MAM model of schizophrenia. In this model, MAM (an anti-mitotic that regulates DNA methylation) is administered to pregnant rats, affecting the brain regions undergoing the most rapid development in the brain of the fetus. The offspring demonstrates behavioural and neuroanatomical phenotypes consistent with that observed in schizophrenics, thus possessing a high face and construct validity (Lodge & Grace, 2009). However, few studies tested the ability of pharmacological compounds to reverse the effect of gestational MAM treatment, meaning that the predictive validity is yet to be determined (S. B. Powell, 2010).

Another neurodevelopmental model includes the post-weaning isolation model. This model utilizes the fact that rats display a defined social structure and create a hierarchy that influences their development. When isolated, rats display neophobia, impaired sensorimotor gating, aggression, cognitive rigidity, reduced prefrontal cortical volume and decreased cortical and hippocampal synaptic plasticity (Fone & Porkess, 2008). These changes are associated with hyperfunction of mesolimbic dopaminergic systems, enhanced presynaptic dopamine and serotonergic function in the nucleus accumbens, hypofunction of mesocortical dopamine and attenuated serotonergic function in the prefrontal cortex and hippocampus (Fone & Porkess, 2008). These are core features of schizophrenia, meaning that this model possesses a high face and construct validity. Again, the predictive validity of the post-weaning isolation model has yet to be determined.

The most promising feature of the neurodevelopmental models however is that the behavioural changes observed appear post-puberty, consistent with the human pathology and thus face validity of this model (Jones et al., 2011; 2012). Moreover, neurodevelopmental models can be combined with other induction categories presenting valuable new insights (Buuse, Garner, & Koch, 2003).

### *Pharmacological models*

The first animal models for schizophrenia were pharmacological models attempting to mimic the idea that dopamine dysregulation with hyperfunction of the mesolimbic dopamine system were underlying the basis of schizophrenia (Murray, Lappin, & Di Forti, 2008).

Amphetamine is believed to be such a drug. Chronic administration indeed induces hyperlocomotion and psychotic-like changes similar to the positive symptoms of schizophrenia (Featherstone, Kapur, & Fletcher, 2007). In contrast, cognitive and negative symptoms are not observed in this model (Featherstone, Rizos, Kapur, & Fletcher, 2008). Together with a low construct and predictive validity the credibility of this model is therefore questionable (Jones et al., 2011; 2012).

However, recent findings show that not only the dopaminergic system is affected in schizophrenics, also the glutamatergic system is dysfunctioned (Konradi & Heckers, 2003). Phencyclidine (PCP), a non-competitive NMDA-receptor antagonist, acts on this system and causes delusions and hallucinations in humans and aggravate these positive symptoms in schizophrenics at low dose (Javitt & Zukin, 1991).

This “glutamate hypothesis of schizophrenia” has been used to develop animal models of schizophrenia by (chronic) administration of PCP in rats. PCP-treatment results in hyperlocomotion (positive symptoms), social behavioral deficit in a social interaction test and enhanced immobility in a

forced swimming test (negative symptoms). Cognitive symptoms of this model include a sensorimotor gating deficit and dysfunctions in several learning and memory tests. Also, all symptoms remain after PCP treatment is stopped, excluding the possibility that the effects are directly caused by PCP itself. Thus, the PCP animal model exhibits a very high face validity (Enomoto, Noda, & Nabeshima, 2007).

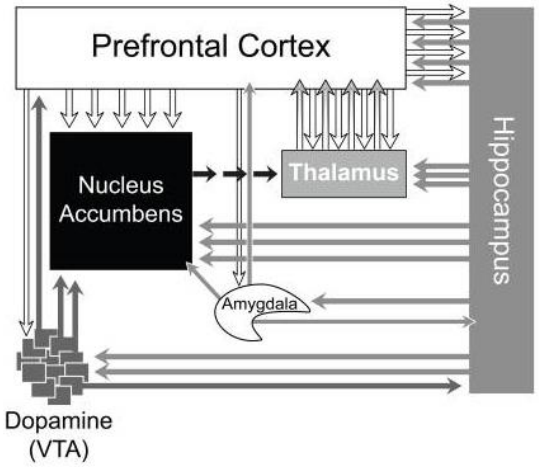
Also, the effects of PCP in monkeys can be reversed by the atypical drug Clozapine, indicating a certain predictive validity as well (Jentsch et al., 1997).

The criticism of chronic PCP models is that it is given to adult rats, which does not have construct validity to the proposed Neonatal PCP treatment provides a solution for this and results in behavioural and neurochemical changes in adulthood. Moreover, these effects are believed to be reversed by antipsychotics such as olanzapine and risperidone, increasing the predictive validity of neonatal PCP treatment. However, these findings are not consistent and should be treated with caution (Jones et al., 2011; 2012).

### *Lesion models*

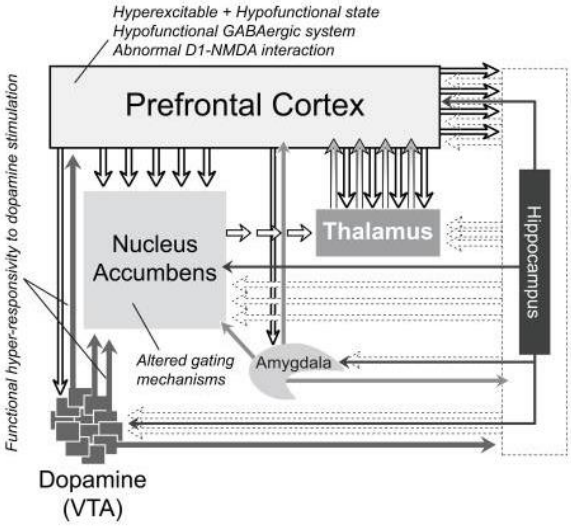
In the early 1990s Lipska and Weinberger developed a new category of induction. Neonatal ventral hippocampal lesion (NVHL) at the 7$^{th}$ day after birth has since then been used comprehensively as a model to study the neuronal changes observed in neurodevelopmental origin of schizophrenia. schizophrenia (O'Donnell, 2011).

Normal




A diagram illustrating major brain circuits thought to be involved in the pathophysiology of schizophrenia. They are anatomically and functionally interconnected, and are crucial in determining appropriate decision making outcomes in response to external stimuli

NVHL




The development and function of multiple brain systems are affected in the NVHL model, including the frontal and medial temporal lobes, the ventral striatum, and the mesocorticolimbic dopamine system. Early developmental insult of the ventral hippocampus has the unique capacity to change function of a multitude of brain regions and may thus capture the complexity of the disease process of schizophrenia.

**Figure 3**

NVHL does not reproduce the hippocampal changes in schizophrenia, as patients do not present a lesion. However, correct timing of the lesion inhibits correct innervation of the hippocampus and results in long-term consequences on synaptic connectivity and function of prefrontal cortex neural circuits (Tseng, Chambers, & Lipska, 2009).

In figure 3 is shown that in the normal development of the brain regions involved in schizophrenia are highly influenced by the hippocampus. When this region is affected (due to a lesion in the NVHL model, but naturally affected in schizophrenia) these brain regions are also affected. This model is therefore promising in studying the neuropathological pathways, making it a model with high contruct validity without the genetic make-up of schizophrenics.

Typical positive, negative, cognitive-

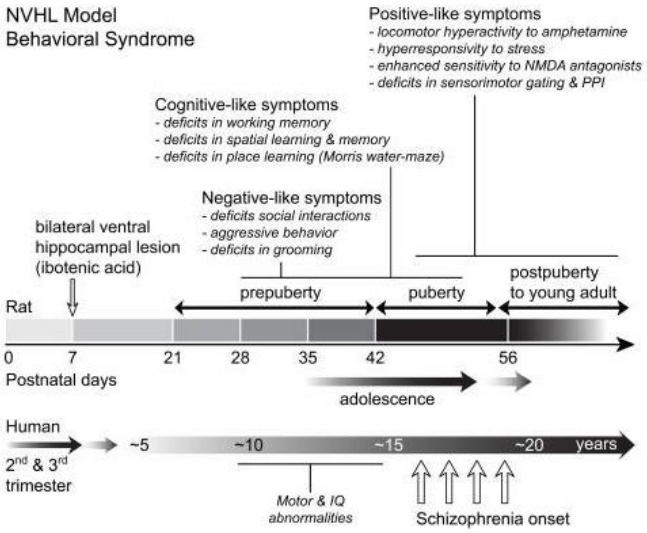



**Figure 4** A timeline of the emergence of behavioral changes following the neonatal ventral hippocampal lesion in the rat and a comparison with the emergence of symptoms in schizophrenia in humans. Developmental ventral hippocampal damage produces a number of dopamine-related behavioral abnormalities that emerge late in adolescence and early adulthood, resembling the delayed onset of psychotic symptoms in schizophrenia. Other NVHL-induced behavioral anomalies, such as negative-like symptoms and cognitive deficits [3, 18, 38–43], occur at both pre- (PD 35) and postpubertal (PD 65) ages, similar to subtle developmental anomalies in humans who later develop schizophrenia, and do not respond to antipsychotic drugs. *(Tseng et al. 2009)*

like symptoms are observed in the NVHL model, along with increased addiction vulnerability, resembling that in schizophrenia. Moreover, the symptoms are expressed progressively in a way very comparable to that of schizophrenic patients, as shown in figure 4. Thus, NVHL-rats provide an important platform to further investigate the pathways leading to the (late) onset of schizophrenia and may lead to preventative strategies (Tseng et al., 2009).

## *Genetic models*

The last category of induction is the category of genetic alterations to simulate the neuropathology of schizophrenia in animals. As mentioned before, many candidate genes have been associated with an increased risk of developing schizophrenia. The majority of genetic models have been developed replicating the changes in mRNA and protein observed in schizophrenia. Several models provide interesting evidence for a high face and construct validity of animal models of schizophrenia. Describing all these models would exceed the scope of this thesis, but are reviewed extensively (O'Tuathaigh, Kirby, Moran, & Waddington, 2010). Therefore, in this thesis only two genetic models will be discussed as an example of the oppurtunities in this research field.

*Dysbindin*

One of the many molecular targets thought to underlie alterations in neurotransmitter release seen in schizophrenia is dysbindin (Jones et al., 2011; 2012). This synaptic protein is thought to be involved in exocytosis, vesicle biogenesis and receptor trafficking involved in neurotransmission (Karlsgodt et al., 2011).

Mutations in the dysbindin gene (DTNBP1) in mice result in memory dysfunction and excitatory neurotransmission abnormalities in the prefrontal and hippocampal networks by inhibition of presynaptic glutamatergic neurotransmission.

These findings are consistent with observations derived from human post-mortem studies that show reduced dysbindin expression in the prefrontal cortex and hippocampus of schizophrenic patients and is linked to the negative symptoms of schizophrenia (Papaleo et al., 2012).

A spontaneous mutant of this gene (known as the *sandy* mouse) has been back-crossed with a C57BL/6J strain resulting in homo- and heterozygous mutants. These mutants display promising alterations in dendritic spines of excitatory asymmetric synapses in the hippocampal CA1 region with narrower synaptic clefts in excitatory junctions, broader postsynaptic densities and a reduced number of larger presynaptic glutamatergic vesicles (Jaaro-Peled, Ayhan, Pletnikov, & Sawa, 2010).

The behavioural components of this model have yet to be investigated thoroughly, but some studies indicate behaviour comparable with schizophrenics in dysbindin mutants. Impaired (spatial) learning and social interaction in several tests is reported (Papaleo et al., 2012).

*APO-SUS/APO-UNSUS*

The second genetic model that will be discussed is the APO-SUS/APO-UNSUS model developed by the group of Ellenbroek en Cools. This model is of great importance for this thesis, because it has many common characteristics with the RLA/RHA rat that will be discussed later.

Apomorphine is a $D_1/D_2$ receptor agonist that induces several dopamine-mediated behaviours, such as locomotor hyperactivity, climbing behaviour, and stereotype grooming, licking and gnawing. Selective breeding of Wistar rats with a high response to these apomorphine-induced behaviours resulted in a strain of susceptible (APO-SUS) rats. On the other hand, Wistar rats with a low susceptibility to apomorphine were used to create a 'mirror' strain (APO-UNSUS) (Ellenbroek & Cools, 2002).

This APO-SUS/APO-UNSUS strain shows how valuable spontaneous genetic variation in strains and breeding lines can be manipulated to research the contribution that interactions between gene and environmental stressors can make to disease (van Loo & Martens, 2007). Indeed, the APO-SUS strain shows remarkable similarity to the schizophrenic phenotype.

The first evidence is derived from the observation that schizophrenic patients are hypersensitive to apomorphine, as of course APO-SUS rats are as well. Also, increased mRNA levels for TH and increased $D_2$ receptor densities have been observed in APO-SUS rats and schizophrenic patients, although in humans these changes may be secondary to antipsychotic drug treatment (Ellenbroek & Cools, 2002).

Second, APO-SUS rats show abnormalities in information processing resulting in a reduced PPI and latent inhibition (Ellenbroek, Geyer, & Cools, 1995). Also, a higher sensitivity to drugs of abuse, such as amphetamine and alcohol, is observed, in line with the high co-morbidity between schizophrenia and drug abuse (Cools, Ellenbroek, Gingras, Engbersen, & Heeren, 1997).

Relatively unique for animal models of schizophrenia is that APO-SUS rats drink relatively less sucrose water when given a free choice. Since this is sometimes regarded as a paradigm of anhedonia, APO-SUS rats display some of the negative symptoms of schizophrenia as well (Ellenbroek & Cools, 2000).

Moreover, next to the CNS similarities, there are also a number of endocrinological and immunological similarities between APO-SUS rats and patients suffering from schizophrenia. Heightened HPA-axis response to stressors, reduction of natural killer cells, relative dominance of $T_{H2}$ cells, reduced susceptibility to adjuvant arthritis and reduced (lung) tumor growth as a result of tumor cell injections are all observations in line with the pathology of schizophrenia (Ellenbroek & Cools, 2002).

All these data taken together provide strong evidence for a high face and construct validity of the APO-SUS strain. The predictive

validity however is not investigated and has yet to be determined (Jones et al., 2011; 2012).

## Roman High Avoidance/Roman Low Avoidance rats as a model of schizophrenia

Another animal model has been created by selectively breeding rats with a high rate of avoidance (Roman High Avoidance or RHA) or a low rate of avoidance (Roman Low Avoidance or RLA) in a shuttle-box two-way avoidance test (Broadhurst & Bignami, 1965).

This animal model initially was not created for the purpose of studying schizophrenia per se. It is widely used to study inter-individual differences in neuroendocrine and behavioural responses to environmental challenges within the context of psychogenetic selection (Steimer & Driscoll, 2005).

However, because RHA rats display behaviours comparable with schizophrenia such as increased impulsivity (Moreno et al., 2010; Steimer & Driscoll, 2003), they are worthwhile investigating. Also, RHA rats show increased dopamine turnover and increased stereotypy in response to apomorphine challenges, indicating a similarity of RHA rats and APO-SUS rats. Finally, RHA rats are characterised by increased dopaminergic activity, impulsivity and compulsivity, in line with symptoms observed in schizophrenia.

On the other hand, RLA rats are characterised by increased leptin and insulin levels and are therefore more likely to increase body weight and develop metabolic diseases naturally (Boersma, Scheurink, Wielinga, Steimer, & Benthem, 2009).

Last year, we studied the effect of the atypical, second generation antipsychotic drug Olanzapine (OLZ). OLZ is an antagonist, primarily acting on the 5-HT$_{2A/C}$ and dopamine receptors and often used in the treatment of schizophrenia. (Bymaster, Hemrick-Luecke, Perry, & Fuller, 1996). However, in humans negative side effects of OLZ treatment include body weight gain and an increased risk of developing diabetes type II as a result of increased food intake, increased leptin levels, enhanced insulin levels and insulin resistance (Graham et al., 2005).

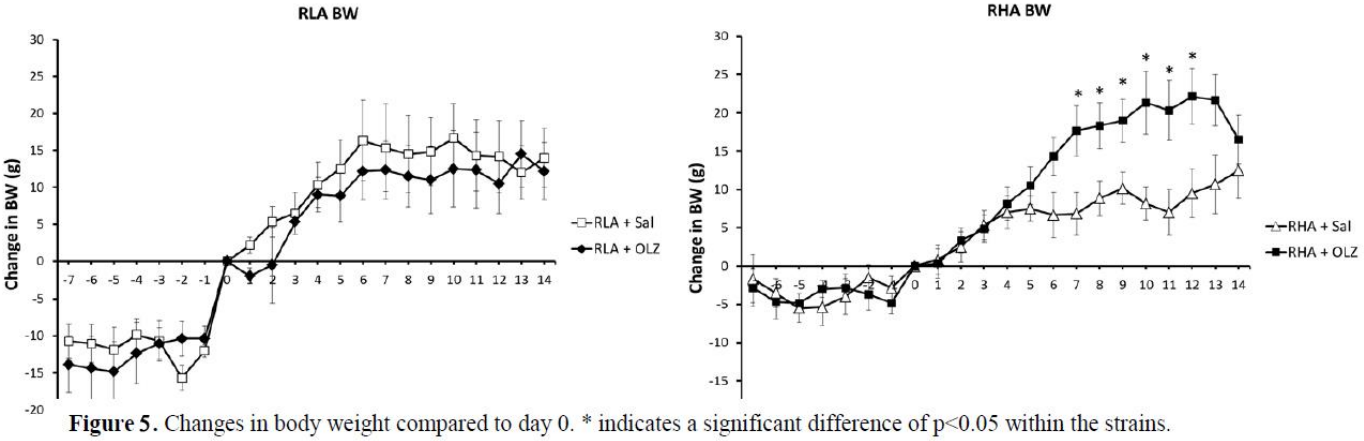



**Figure 5.** Changes in body weight compared to day 0. * indicates a significant difference of $p<0.05$ within the strains.

To study the underlying mechanisms, simultaneously including inter-individual variation in our experimental design, we administered OLZ to RHA/RLA rats for two weeks. The effects of OLZ on locomotor activity, metabolism and reproductive function were studied. Due to the increased sensibility to metabolic effects, we expected a higher effect of OLZ in the RLA strain, compared to the RHA rats.

However, as shown in figure 5, the RHA rats were more susceptible to OLZ-induced weight gain, whereas the RLA rats naturally are more vulnerable for metabolic anomalies. This indicates the value of inter-individual variation in research set-up once again (Koolhaas et al., 2010).

Possible explanations of this observation can originate from the differences in receptor expression between the two strains. RHA rats are characterised by increased dopamine receptor expression in the brain. Since OLZ is a dopamine receptor antagonist, it has more potential of acting in the RHA strain, compared to the RLA strain. However, the mechanisms by which increased antagonism on dopamine receptors leads to increased body weight gain have to be elucidated.

Moreover, we studied the estrous cycle of the rats, suggesting a normal estrous cycle in RLA rats, whereas in the RHA strain animals tend to display a disturbed estrous cycle after OLZ treatment (data not shown). Since metabolic variations are observed during the estrous cycle, disruption (together with reduced locomotor activity) as a result of OLZ treatment can in part explain the weight gain differences observed.

In conclusion, RHA rats provide interesting evidence of several aspects of schizophrenia, raising the possibility of being an interesting model of schizophrenia. Their increased response to Olanzapine may contribute to a certain predictive validity as well. However, this is still very uncertain and has to be further investigated.

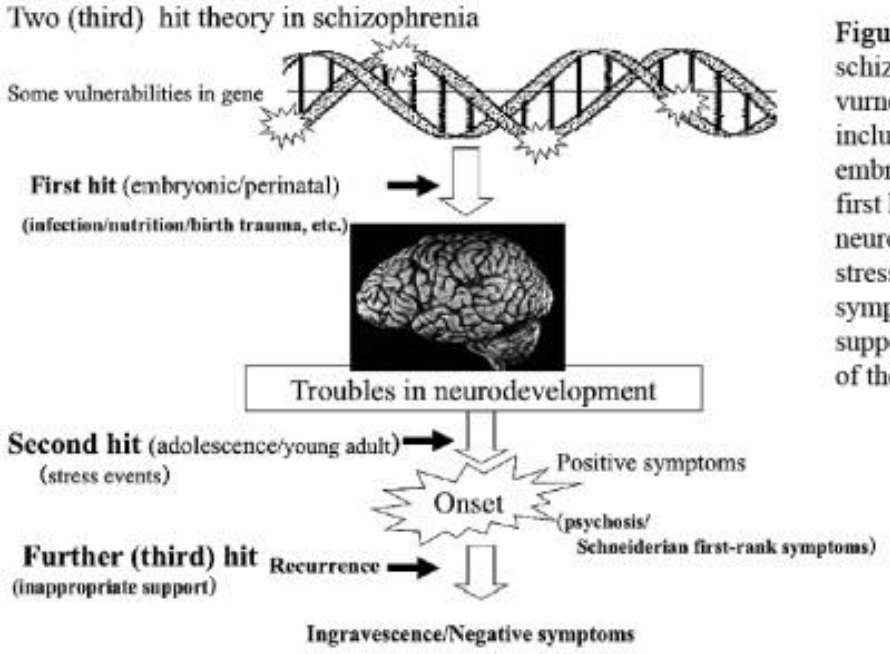



**Figure 6.** The two (third) hits theory of schizophrenia's illness course. Some vurnerabilities exist in genes. Some impacts, including infection or birth trauma etc. in the embryonic/perinatal period on the brain are the first hit. These factors may later induce neurodevelopmental dysfunction. Psychological stressors will trigger the onset of schizophrenic symptoms as a second hit. Lack of appropriate support will lead to progression of the pathology of the illness as a third hit. *(Iritani, 2013)*

## Two (third) hit hypothesis of schizophrenia

To create an animal model that matches the (neuro)pathology of schizophrenia best, profound studies on onset and development in humans fed back to animal studies create a selection cycle that improves both animal models, knowledge of the disorder and ultimately treatment in humans. This already resulted in a theory pointed out by (Iritani, 2013).

The two (third) hit hypothesis is already known from the development of cancer and has been proposed for schizophrenia as well (Maynard, Sikich, Lieberman, & LaMantia, 2001). It states that individuals with a certain genetic vulnerability that are exposed to stress during gestation (first hit), will develop abnormalities in the brain. When a second hit (traumatic event or stress) strikes this person, it will develop the positive symptoms. A third hit in the form of inappropriate support of this person will trigger ingravescence and negative symptoms (figure 6).

This theory is supported by studies showing a twofold risk of developing schizophrenia when abnormalities during the perinatal period occur (Lewis & Levitt, 2002), and even a 2.6 fold when a child has a low body weight at birth (Kunugi et al., 1997)(Lewis & Levitt, 2002). Also, the Dutch famine, a typical period of malnutrition of the unborn, was followed by an increased risk of developing schizophrenia (Roseboom, Painter, van Abeelen, Veenendaal, & de Rooij, 2011). The following troubles in neuronal development are demonstrated as well (Benes & Berretta, 2001). The third hit remains a matter of debate, but is proposed to be essential for developing the negative symptoms of schizophrenia, as the positive symptoms are commonly observed prior to the negative symptoms (Iritani, 2013).

Evidence of postnatal stress in animals affecting phenotype comes from unpublished data by Gretha Boersma. As shown in figure 7, postnatal stress has great influence on adult

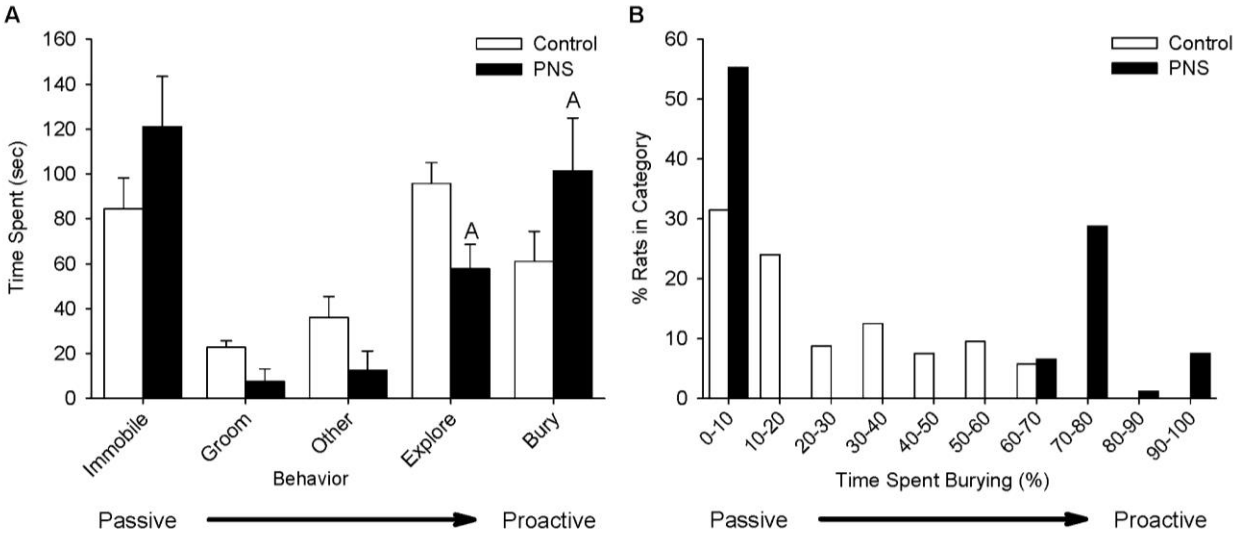



**Figure 7.** Postnatal stress (PNS) changes the distibution of passive and proctive animals to more extreme phenotypes compared to the control animals. *(Gretha Boersma, unpublished data)*

behaviour compared to control animals, resulting in a shift from a more or less normal distribution of passive and proactive animals to a rather extreme pattern of large groups of highly passive or proactive animals.

This correlates with the idea that pre- or postnatal stress induces changes that expose an individual's "true nature" with regard to its genetic make-up. In contrast to a stable, stress-free environment and gestation without complications, pushing the population to a normal distribution.

## Discussion and conclusion

Overall, finding an animal model that suits all the validities and displays the exact development pattern as seen in schizophrenic patients is an illusion. However, more and more models increase face, construct and predictive validity. Though this last aspect has yet to be investigated in many models.

Evidence of inter-individual studies has shown great importance of including this factor in research. Schizophrenic studies should therefore focus more on the individual differences, instead of a common concept of the development of the disorder. Differences such as the RLA/RHA strain could provide interesting data and should therefore be included in schizophrenic research.

Many studies show promising results of the four induction categories with regard to the validities described in this thesis separately. However, treatment of schizophrenia is always too late and further knowledge of this disorder is needed, highlighting the need for an animal model that exhibits as many common characteristics with schizophrenia as possible. Therefore, combination of the four induction strategies would be very interesting.

A genetic model (APO-SUS/UNSUS or RHA/RLA) could be stressed prenatally (gestational MAM representing the first hit) and isolated (social isolation representing the second hit) or treated with PCP to induce the positive symptoms. This is of course only one possibility of the countless combinations conceivable with this approach.

Therefore, a lot of research has to be done in order to create an animal model possessing a high face, construct and predictive validity for schizophrenia. However, on the condition that findings in clinical studies are carefully transferred to the fundamental research performed in animal studies, ultimately we will be able to increase and improve our knowledge and preventative strategies to this devastating disorder.

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## References


1. Akbarian, S. (2010). Epigenetics of schizophrenia. *Current Topics in Behavioral Neurosciences, 4*, 611-628.
2. Andreasen, N. C. (1995). Symptoms, signs, and diagnosis of schizophrenia. *Lancet, 346*(8973), 477-481.
3. Andreasen, N. C., & Olsen, S. (1982). Negative v positive schizophrenia. definition and validation. *Archives of General Psychiatry, 39*(7), 789-794.
4. Benes, F. M., & Berretta, S. (2001). GABAergic interneurons: Implications for understanding schizophrenia and bipolar disorder. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology, 25*(1), 1-27.
5. Boersma, G. J., Scheurink, A. J. W., Wielinga, P. Y., Steimer, T. J., & Benthem, L. (2009). The passive coping roman low avoidance rat, a non-obese rat model for insulin resistance. *Physiology & Behavior, 97*(3-4), 353 - 358.
6. Braff, D. L. (1990). Sensorimotor gating and SchizophreniaHuman and animal model studies. *Archives of General Psychiatry, 47*(2), 181.
7. Broadhurst, P. L., & Bignami, G. (1965). Correlative effects of psychogenetic selection: A study of the roman high and low avoidance strains of rats. *Behaviour Research and Therapy, 3*, 273-280.
8. Bushnell PJ, S. B. (2009). Assessing attention in rats. In Buccafusco JJ (Ed.), *Methods of behavior analysis in neuroscience* (2nd ed., ) CRC Press.
9. Buuse, M., Garner, B., & Koch, M. (2003). Neurodevelopmental animal models of schizophrenia: Effects on prepulse inhibition. *Current Molecular Medicine, 3*(5), 459 - 471.
10. Bymaster, F. P., Hemrick-Luecke, S. K., Perry, K. W., & Fuller, R. W. (1996). Neurochemical evidence for antagonism by olanzapine of dopamine, serotonin, a1-adrenergic and muscarinic receptors in vivo in rats. *Psychopharmacology, 124*(1-2), 87- 94.
11. Cools, A. R., Ellenbroek, B. A., Gingras, M. A., Engbersen, A., & Heeren, D. (1997). Differences in vulnerability and susceptibility to dexamphetamine in nijmegen high and low responders to novelty: A dose-effect analysis of spatio-temporal programming of behaviour. *Psychopharmacology, 132*(2), 181-187.
12. Ellenbroek, B. A., & Cools, A. R. (2000). Animal models for the negative symptoms of schizophrenia. *Behavioural Pharmacology, 11*(3-4), 223-233.
13. Ellenbroek, B. A., & Cools, A. R. (2002). Apomorphine susceptibility and animal models for psychopathology: Genes and environment. *Behavior Genetics, 32*(5), 349-361.
14. Ellenbroek, B. A., Geyer, M. A., & Cools, A. R. (1995). The behavior of APO-SUS rats in animal models with construct validity for schizophrenia. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 15*(11), 7604-7611.
15. Enomoto, T., Noda, Y., & Nabeshima, T. (2007). Phencyclidine and genetic animal models of schizophrenia developed in relation to the glutamate hypothesis. *Methods and Findings in Experimental and Clinical Pharmacology, 29*(4), 291-301.
16. Featherstone, R. E., Kapur, S., & Fletcher, P. J. (2007). The amphetamine-induced sensitized state as a model of schizophrenia. *Progress in Neuro-Psychopharmacology & Biological Psychiatry, 31*(8), 1556-1571.
17. Featherstone, R. E., Rizos, Z., Kapur, S., & Fletcher, P. J. (2008). A sensitizing regimen of amphetamine that disrupts attentional set-shifting does not disrupt working or long-term memory. *Behavioural Brain Research, 189*(1), 170-179.
18. Fone, K. C. F., & Porkess, M. V. (2008). Behavioural and neurochemical effects of post-weaning social isolation in rodents—Relevance to developmental neuropsychiatric disorders. *Neuroscience &*

*Biobehavioral Reviews, 32*(6), 1087 - 1102.
19. G.F. Koob. (2012). Animal models of psychiatric disorder. In B. a. S. Aminoff (Ed.), *Handbook of clinical neurology* (pp. 145-150) Elsevier.
20. Graham, K. A., Perkins, D. O., Edwards, L. J., Barrier, R. C.,Jr, Lieberman, J. A., & Harp, J. B. (2005). Effect of olanzapine on body composition and energy expenditure in adults with first-episode psychosis. *The American Journal of Psychiatry, 162*(1), 118-123.
21. Harrison, P. J., & Weinberger, D. R. (2005). Schizophrenia genes, gene expression, and neuropathology: On the matter of their convergence. *Molecular Psychiatry, 10*(1), 40-68;
22. Holmes, A., & Wellman, C. L. (2009). Stress-induced prefrontal reorganization and executive dysfunction in rodents. *Neuroscience & Biobehavioral Reviews, 33*(6), 773 - 783.
23. Iritani, S. (2013). What happens in the brain of schizophrenia patients?: An investigation from the viewpoint of neuropathology *Nagoya Journal of Medical Science, 75*(1-2), 11-28.
24. Jaaro-Peled, H., Ayhan, Y., Pletnikov, M. V., & Sawa, A. (2010). Review of pathological hallmarks of schizophrenia: Comparison of genetic models with patients and nongenetic models *Schizophrenia Bulletin, 36*(2), 301-313.
25. Javitt, D. C., & Zukin, S. R. (1991). Recent advances in the phencyclidine model of schizophrenia. *The American Journal of Psychiatry, 148*(10), 1301-1308.
26. Jentsch, J. D., Redmond, D. E.,Jr, Elsworth, J. D., Taylor, J. R., Youngren, K. D., & Roth, R. H. (1997). Enduring cognitive deficits and cortical dopamine dysfunction in monkeys after long-term administration of phencyclidine. *Science (New York, N.Y.), 277*(5328), 953-955.
27. Jones, C., Watson, D., & Fone, K. (2011; 2012). Animal models of schizophrenia. *British Journal of Pharmacology, 164*(4), 1162 - 1194.
28. Kamiya, A., Kubo, K., Tomoda, T., Takaki, M., Youn, R., Ozeki, Y., . . . Sawa, A. (2005). A schizophrenia-associated mutation of DISC1 perturbs cerebral cortex development. *Nature Cell Biology, 7*(12), 1167-1178.
29. Karlsgodt, K. H., Robleto, K., Trantham-Davidson, H., Jairl, C., Cannon, T. D., Lavin, A., & Jentsch, J. D. (2011). Reduced dysbindin expression mediates N-methyl-D-aspartate receptor hypofunction and impaired working memory performance. *Biological Psychiatry, 69*(1), 28-34.
30. Konradi, C., & Heckers, S. (2003). Molecular aspects of glutamate dysregulation: Implications for schizophrenia and its treatment. *Pharmacology & Therapeutics, 97*(2), 153-179.
31. Koolhaas, J. M., de Boer, S. F., Coppens, C. M., & Buwalda, B. (2010). Neuroendocrinology of coping styles: Towards understanding the biology of individual variation. *Frontiers in Neuroendocrinology, 31*(3), 307-321.
32. Kunugi, H., Vallada, H. P., Sham, P. C., Hoda, F., Arranz, M. J., Li, T., . . . Collier, D. A. (1997). Catechol-O-methyltransferase polymorphisms and schizophrenia: A transmission disequilibrium study in multiply affected families. *Psychiatric Genetics, 7*(3), 97-101.
33. Lannfelt, L., Folkesson, R., Mohammed, A. H., Winblad, B., Hellgren, D., Duff, K., & Hardy, J. (1993). Alzheimer's disease: Molecular genetics and transgenic animal models *Behavioural Brain Research, 57*(2), 207-213.
34. Lewis, D. A., & Levitt, P. (2002). Schizophrenia as a disorder of neurodevelopment. *Annual Review of Neuroscience, 25*, 409-432.
35. Lodge, D. J., & Grace, A. A. (2009). Gestational methylazoxymethanol acetate administration: A developmental disruption model of schizophrenia. *Behavioural Brain Research, 204*(2), 306-312.
36. Lubow, R. E., & Gewirtz, J. C. (1995). Latent inhibition in humans: Data,

theory, and implications for schizophrenia. *Psychological Bulletin, 117*(1), 87-103.
37. Maher, B. A. (1983). A tentative theory of schizophrenic utterance. *Progress in Experimental Personality Research, 12*, 1-52.
38. Maynard, T. M., Sikich, L., Lieberman, J. A., & LaMantia, A. S. (2001). Neural development, cell-cell signaling, and the "two-hit" hypothesis of schizophrenia. *Schizophrenia Bulletin, 27*(3), 457-476.
39. McClellan, J. M., Susser, E., & King, M. C. (2007). Schizophrenia: A common disease caused by multiple rare alleles. *The British Journal of Psychiatry : The Journal of Mental Science, 190*, 194-199.
40. McGonigle, P. (2013). Animal models of CNS disorders. *Biochemical Pharmacology,*
41. Moreno, M., Cardona, D., Gómez, M. J., Sánchez-Santed, F., Tobeña, A., Fernández-Teruel, A., . . . Flores, P. (2010). Impulsivity characterization in the roman high- and low-avoidance rat strains: Behavioral and neurochemical differences. *Neuropsychopharmacology,*
42. Murray, R. M., Lappin, J., & Di Forti, M. (2008). Schizophrenia: From developmental deviance to dopamine dysregulation. *European Neuropsychopharmacology : The Journal of the European College of Neuropsychopharmacology, 18 Suppl 3*, S129-34.
43. O'Donnell, P. (2011). Cortical disinhibition in the neonatal ventral hippocampal lesion model of schizophrenia: New vistas on possible therapeutic approaches. *Pharmacology & Therapeutics,*
44. O'Tuathaigh, C. M., Kirby, B. P., Moran, P. M., & Waddington, J. L. (2010). Mutant mouse models: Genotype-phenotype relationships to negative symptoms in schizophrenia. *Schizophrenia Bulletin, 36*(2), 271-288.
45. Owen, M. J., Williams, N. M., & O'Donovan, M. C. (2003; 2004). The molecular genetics of schizophrenia: New findings promise new insights. *Molecular Psychiatry, 9*(1), 14 -27.
46. Papaleo, F., Yang, F., Garcia, S., Chen, J., Lu, B., Crawley, J. N., & Weinberger, D. R. (2012). Dysbindin-1 modulates prefrontal cortical activity and schizophrenia-like behaviors via dopamine/D2 pathways. *Molecular Psychiatry, 17*(1), 85-98.
47. Picchioni, M. M., & Murray, R. M. (2007). Schizophrenia *BMJ (Clinical Research Ed.), 335*(7610), 91-95.
48. Powell, S. B. (2010). Models of neurodevelopmental abnormalities in schizophrenia. In N. R. Swerdlow (Ed.), *Behavioural neurobiology of schizophrenia and its treatment* (pp. 435-484) Springer.
49. Powell, C. M., & Miyakawa, T. (2006). Schizophrenia-relevant behavioral testing in rodent models: A uniquely human disorder? *Biological Psychiatry, 59*(12), 1198-1207.
50. Reader, T. A., & Dewar, K. M. (1999). Effects of denervation and hyperinnervation on dopamine and serotonin systems in the rat neostriatum: Implications for human parkinson's disease. *Neurochemistry International, 34*(1), 1-21.
51. Roberts, A. C., & Wallis, J. D. (2000). Inhibitory control and affective processing in the prefrontal cortex: Neuropsychological studies in the common marmoset. *Cerebral Cortex (New York, N.Y.: 1991), 10*(3), 252-262.
52. Roberts, G. W., & Bruton, C. J. (1990). Notes from the graveyard: Neuropathology and schizophrenia. *Neuropathology and Applied Neurobiology, 16*(1), 3-16.
53. Roseboom, T. J., Painter, R. C., van Abeelen, A. F., Veenendaal, M. V., & de Rooij, S. R. (2011). Hungry in the womb: What are the consequences? lessons from the dutch famine *Maturitas, 70*(2), 141-145.
54. Steimer, T., & Driscoll, P. (2003). Divergent stress responses and coping styles in psychogenetically selected roman high-(RHA) and low-(RLA) avoidance rats: Behavioural, neuroendocrine and developmental

aspects. *Stress (Amsterdam, Netherlands), 6*(2), 87-100.
55. Steimer, T., & Driscoll, P. (2005). Inter-individual vs line/strain differences in psychogenetically selected roman high-(RHA) and low-(RLA) avoidance rats: Neuroendocrine and behavioural aspects. *Neuroscience and Biobehavioral Reviews, 29*(1), 99-112.
56. Swerdlow, N. R., & Geyer, M. A. (1998). Using an animal model of deficient sensorimotor gating to study the pathophysiology and new treatments of schizophrenia. *Schizophrenia Bulletin, 24*(2), 285-301.
57. Tseng, K. Y., Chambers, R. A., & Lipska, B. K. (2009). The neonatal ventral hippocampal lesion as a heuristic neurodevelopmental model of schizophrenia. *Behavioural Brain Research, 204*(2), 295-305.
58. Tsuang, M. (2000). Schizophrenia: Genes and environment *Biological Psychiatry, 47*(3), 210 - 220.
59. van Loo, K. M., & Martens, G. J. (2007). Identification of genetic and epigenetic variations in a rat model for neurodevelopmental disorders. *Behavior Genetics, 37*(5), 697-705.
60. van Os, J., & Kapur, S. (2009). Schizophrenia *Lancet, 374*(9690), 635-645.
61. Young, J. W., Zhou, X., & Geyer, M. A. (2010). Animal models of schizophrenia. *Current Topics in Behavioral Neurosciences, 4*, 391-433.
62. Zakzanis, K. K., & Hansen, K. T. (1998). Dopamine D2 densities and the schizophrenic brain. *Schizophrenia Research, 32*(3), 201 - 206.

# Appendix

## Common side effects of antipsychotic drugs

**First generation antipsychotics**

- Extrapyramidal effects:
  - Dystonia
  - Pseudoparkinsonism
  - Akathisia
  - Tardive dyskinesia
- Sedation
- Hyperprolactinaemia
- Reduced seizure threshold
- Postural hypotension
- Anticholinergic effects:
  - Blurred vision
  - Dry mouth
  - Urinary retention
- Neuroleptic malignant syndrome
- Weight gain
- Sexual dysfunction
- Cardiotoxicity (including prolonged QTc)

**Second generation antipsychotics**

- Olanzapine:
  - Weight gain
  - Sedation
  - Glucose intolerance and frank diabetes mellitus
  - Hypotension
- Risperidone:
  - Hyperprolactinaemia
  - Hypotension
  - Extrapyramidal side effects at higher doses
  - Sexual dysfunction
- Amisulpiride:
  - Hyperprolactinaemia
  - Insomnia
  - Extrapyramidal effects
- Quetiapine:
  - Hypotension
  - Dyspepsia
  - Drowsiness

**Clozapine**

- Sedation
- Hypersalivation
- Constipation
- Reduced seizure threshold
- Hypotension and hypertension
- Tachycardia
- Pyrexia
- Weight gain
- Glucose intolerance and diabetes mellitus
- Nocturnal enuresis
- Rare serious side effects:
  - Neutropenia (93%)
  - Agranulocytosis (0.8%)
  - Thromboembolism
  - Cardiomyopathy
  - Myocarditis
  - Aspiration pneumonia


  - Myocarditis
  - Aspiration pneumonia