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Neurosurgery in Schizophrenic Mouse (A Proposal Project)

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Abstract

Schizophrenia is an illness that devastates not only the life of patients, but also of friends, family, and the community. Schizophrenia is characterized by bizarre behavior and complex negative and positive symptoms. Researchers estimate that, approximately one third of the conventional antipsychotics fail to achieve a good response and this means increased suffering of these patient as well as increased numbers of suicide attempts [1]. Furthermore some of antipsychotics medications such as thioridazine and haloperidol have extra pyramidal side effects. While other medications like olanzapine and other 5-HT3 antagonists are associated with increase body weight [2,3].

In this paper, we present our efforts to achieve a suitable solution to modulate the positive symptoms of refractory chronic schizophrenia in special genetically modulated mice. Currently, there are many mutant genes used to study schizophrenia but the most susceptible genes are these encoding neuregulin (NRG1) [4,5]. This mice model will be subjected to neurosurgical procedure of deep brain stimulation (DBS) under MRI control, with aid of steriotaxis frame. We proposed that DBS of nucleus accumbens (NAc) or hippocampus can lead to improve positive symptoms of schizophrenia depending on the basis of dopamine hyperactivity theory of schizophrenia [6].

This proposal study can followed by other studies. For instance, we can observe our results directly by using the aid of fMRI or positron emission tomography (PET) to compare the metabolic process in schizophrenic mouse brain with these of the control group [7]. Regarding the improvement of the mice condition, we can also use biochemical assays to define the level of neurotransmitters related to schizophrenia. Our last aim is to study the effect of NAc DBS on dopamenergic (DA) systems in schizophrenic patient through wide clinical trials [8].

Keywords: Schizophrenia; Mouse; NRG1 models; neurosurgery; steriotaxis; Nucleolus accumbens

Introduction

Incidence and clinical features of schizophrenia

Schizophrenia is a mental disease which can occur at any age but mostly during adolescence and early adulthood. Schizophrenia is more prevalent than diabetes or Alzheimer and it affects about 1% of the population. Thus, it can be considered as one of the most disabling diseases all over the world [9]. There is evidence of some relation between socioeconomic status, race, alcoholism, drugs addiction and schizophrenia prevalence. There is also evidence of increased the prevalence of the disease in urban areas [10]. The clinical features are different from one person to another but they may share common features. Some scientists are divide the symptoms of the schizophrenia into negative and positive symptoms. The negative symptoms related with loss of wellbeing, depression, loss of pleasure and decrease memory while the positive signs related with delusion, hallucination and disturbance of thinking processes [11].

There is also abnormal behavior that seems inappropriate to specific situation or delusions by which the patient beliefs some powers directed his life or ask him to do special orders. Hearing, seeing, smelling or feeling hallucinations can also occur. The patient may have a speech defect in form of unconnected sentences or incomprehensible speech (Schneider's first-rank symptoms [12]. All these symptoms are called positive symptoms. Beside that there are also negative symptoms in form of loss of interest and decreased emotion and motive [13]. Symptoms might revile diminish or damage of prefrontal cortex and its connection with parietal and limbic centers [14]. Impairment of memory and cognition can occur in all schizophrenic patients and do not depend upon schizophrenic types or the severity of the symptoms [15].

Cognitive impairment might associated with increase in size of the ventricle in schizophrenic patients [16]. Different types of schizophrenia can be detected according to special and important feature in every schizophrenic patient; Paranoid schizophrenia is the most common one. The people with this types of schizophrenia has thoughts reflect conspiracy and persecution beside the predominant features of delusion and auditory hallucination. These types of schizophrenia occur in late adulthood. Catatonic type has features adjoined with movement disturbance and diminished to the extent that the movement stops (catatonic stupor) or shows more activity (catatonic excitement), the pose might also effected (Waxy flexibility) or takes another forms like repeated what the other persons say (echolalia) or by repeated his movement (echopraxia). In disorganized schizophrenia, the patient has less hallucination and delusion symptoms but he has delay and obvious disturbance in daily activities like bathing, dressing or brushing teeth. The subtype of schizophrenia is not always easy to defined and the symptoms not obvious to categorize in one of the previous subclasses and so called Undifferentiated subtype . By this sub type the symptoms can be changed with time. If the symptoms of schizophrenia such as delusion or hallucination decreased comparing with acute phase of the disease but not disappear completely then the patient has residual subtype [17].

Schizophrenic Patient should differentiate from schizotype personality disorders. This type of personality present mostly among schizophrenic family members and has high risk of the disease but genetically might not affiliated with schizophrenia according to DSM-111 criteria [18].

Physiopathology

Recently many genes in relation with schizophrenia have been identified: (including DISC1, RGS4, neuregulin, dysbindin, G72, COMT and GRM3). These genes might responsible for the molecular and structural changes in brain of schizophrenic patient. Such changes found in glial and neuronal cells as well as in synaptic junctions of dorsolateral prefrontal and hippocampus region of the brain. There is strong evidence of converge network action of all these genes on the brain neurotransmitter like GABA, NMDA receptors to induce alteration in the brain microstructure map [19].

There are cascade of molecular disturbance in neurotransmitters including dopamine glutamate, and GABA neurotransmission, in addition to genetic, epigenetic and environmental factors. All may play an important role in the appearance of the disease [15]. Drugs like amphetamine might induce psychosis like schizophrenia due to interaction with dopamine mechanism [20].

Studies of neuronal connections in various rat models revealed an extensive disturbance in neuronal network between limbic system and basal ganglia including: subiculum, amygdala and nucleus accumbens, There is also some changes in the ascending dopaminergic projection to the accumbens nucleus. This is supported by study of the dopamine agonist effects of amphetamine in acute and chronic schizophrenics [21].

Schizophrenia related theories

There are many theories behind the etiology of schizophrenia such as: Developmental theory, neurotransmitters disturbance theory, genetic mutation, disturbance in immune system, oxidative stress and inflammatory theory [22,23].

Other theories like: The effect of the antioxidant deficiency and its relation with schizophrenia. Such abnormalities might induce deficiency in the energy productions by mitochondria. Therefore the treatment schizophrenic mice by antioxidant food like (N-acetyl cysteine) might improve the condition [22].

Some scientists suggest that the disturbance of the gut microbes may have association with of brain development in early life through interference with the integrity of the gut epithelial and liver function. Mice with normal flora or germ free mice might doing better with anxiety behavior tests like light –dark box than their gut infected colleagues [24].

Currently study of some postmortem cases of chronic schizophrenics denuded the presence of specific types of the APP gene mutation (codon 717 in exon 17). This gene is responsible for synthesis of amyloidal precursor protein in Alzheimer disease. The presence of such protein which is related with dementia might explain the cognitive defects that occur in the later stage of chronic schizophrenic as part of the degenerative Sequencing [25].

Other hypothesis divulges the relation of immunity system disturbance in schizophrenia pathogenesis, depending on the effect of the cytokines on the microglia and astrocyte activation. There is some evidence of increase activity of the dopaminergic pathway in schizophrenia wit out increase number of the dopamine receptor and this might unthread the role of auto antibodies receptor interactions [26].

There is evidence of elevation of anti influenza antibodies in the maternal serum during the 2nd term of the pregnancy. Hence the incidence of the schizophrenia increases during the influenza epidemics in winter and spring [27]. Other studies suggest that no direct connection between the flue epidemics and schizophrenia other than the diagnostic psychiatric data of the hospital [28].

Monozygotic twins studies may provide clues to analyze the connection between the neurodevelopmental theory, genetic factors and the environmental role in schizophrenia (the changes in the metabolic rates of the neurotransmitters) [29]. By using restriction landmark genome scanning, we can achieve direct method to detect the end labeling of DNA segment. These landmarks spots refer to possibility of the methylation status at one or more site. Genomic printing, epigenetic modification (methylation/demethylation) process and others like deletion translocation may explain the heterogeneity of the genetic in the twins and appearance of the clinical features of schizophrenia [30].

Neuroreceptors

1-Glutamate neurotransmitter through N-methyl-D-aspartic acid (NMDA) receptors which are major excitatory receptors to the dorsolateral prefrontal cortex (DLPFC) and can cause both positive and negative symptoms in schizophrenia. The reduction of memory working might occur via alteration of the glutamate receptor binding, transcription of protein, mRNA or decrease of related enzyme glutamate carboxy peptidase (GCP ll). This enzyme has degradation activity for N-acetyl aspartyl glutamate (NAAG), which is a reversible antagonist of NMDA receptor and eventually decrease the activity of NMDA receptor [15].

The dysfunction of the glutamatergic receptor in schizophrenia is one of the important theory to explain the pathophysiology of the disease in Japanese mice model (GRM3) [31]. Furthermore the effect of phencyclidine (NMDA-sensitive glutamate receptor antagonist) might play important role to explore the relation between the glutamatergic hypo activity in hippocampus region and the anterior cingulated cortex in schizophrenic brain [32]. The glutamate hypothesis and schizophrenia suggest 3 ways of glutamate function: Glutamate excitotoxicity (neurodevelopmental theory), Glutamate excitotoxicity (neurodegenerative theory) and NMDA hypo function theory [33].

In relation with our interpretation, the gene for neuregulin 1 (NRG1) has been confirmed to be associated with schizophrenia by influencing N-methyl-D-aspartate (NMDA) receptor. NRG1 induce the activation of tyrosine kinase phosphorylation of erbB4, which increase the interaction of erbB4-PSD95 and decrease the activation of NMDA receptor [15].

Many evidence shown variable glutamatergic neurotransmission disturbance in schizophrenic patient. For instance the D-amino-acid-oxidase-activator (DAOA, former G72) gene is one of the important tool to study schizophrenia, shown that DAOA activates D-amino-acid-oxidase (DAAO) and then destruct D-serine, which is an important agonist of the glutamatergic N-methyl-D-aspartate (NMDA) receptors. It was also shown that D-serine has an important effect to resolve the symptoms of schizophrenia. Furthermore, the medications

that have antagonistic effects at the NMDA receptors can exacerbate the symptoms of schizophrenia. Therefore, it has been suggested that DAAO-DAOA-complex up regulation has degradation effect of D-serine hence schizophrenic symptoms can developed [34].

2-The dopamine neurotransmitters postmortem evidence, using positron emission tomography and pharmacological studies of amphetamine suggest the presence of strong relation between the activation of dopamine receptor (DA) agonist with schizophrenia. There is some suggestion of low DA activity in prefrontal area induced by over activity of DA in mesolimbic region [35]. The presence of DA hyperactivity in schizophrenia comes in contrast with Parkinson's disease which shows dopamine depletion in striatal region especially in putamen (caudal part) and caudate nucleus (dorsal rostral part) which explain the motor deficit in Parkinsonism disease [36]. The hypothesis of the dopamine hyperactivity in schizophrenia is the most dominant one and relay on D2 receptor, although both D2, D3 shown increase in postmortem brain specimens. D4 receptor also not excluded and shown six folds elevation in comparison with D2, D3 which shown increase by only 10% in schizophrenic brain [37].

The deficient of DA agonist receptor in prefrontal area might explain the memory defect in schizophrenia, while the increase activity of DA might relate with the psychosis symptoms. The belief that schizophrenic symptoms mostly due to disturbance of dopamine receptor seen in amphetamine psychosis side affect which mimic paranoid schizophrenia. These symptoms are reduced by neuroleptic drugs due to blocking the dopamine receptor and thus decrease the activation of adenyl cyclase in corpus stratum [12].

The postmortem studies of schizophrenic patient showed decreased in the level of DA in frontotemporal area of the brain. This reduction of DA is related with the impairment of the memory working and reduction of cognitive performance. This is also supported by increase the level of DA degrading enzyme, Catechol- O- methyltransferase (COMT), in sub cortical level and decreases its level in DLPFC area [12].

Schizophrenic patients manifested sensory oversensitivity (pre pulse inhibition of startle responses) which might explained according to the basic of stimulation overload theory: impaired central nervous system inhibition (sensorimotor gating) that conduct to cognitive fragmentation (The increasing of dopamine activity level in nucleus accumbens causing sensorimotor gating failure) [38].

3- GABA neurotransmitters is the major inhibitory transmitter in the brain and shown a strong relation to the memory working impairment in schizophrenia due to decrease its level in DLPFC region. Increase GABA deficit was suggested as a reliable reason of appearance the psychosis symptoms in schizophrenic patients. The using of iomazenil, an antagonist of the benzodiazepine receptor, might support the hypothesis of GABA- receptor deficiency in schizophrenic patient [39].

The postmortem studies shown that the decrease of glutamic acid decarboxylase (*GAD1*), the major determinant of GABA levels, and GABA membrane transporter (GAT1), a protein responsible for reuptake of released GABA into nerve terminals in schizophrenic patient might adjoined memory impairment in prefrontal temporal region of these patients [15].

Reelin is a secretory protease and is one of the important molecular findings in schizophrenia. Reduction of cortical reelin expression can be used as marker for detection schizophrenia. A reduction of reelin expression has been found in the dorsolateral prefrontal cortex, hippocampal and dentate gyrus, near hippocampal fissure in schizophrenia brain. These results might refer to the contribution of reelin with the synaptic pathology of γ-aminobutyric acid (GABA)-ergic neurons and might play major roles in neurodevelopment and plasticity of this synapse [40].

The cartridges (chandelier cell axon terminals) have regulatory role to pyramidal cell, as they have inhibitory input to pyramidal cell. The number of these cells are decrease by about 40% in dorsolateral prefrontal cortex of schizophrenic brain. Therefore these cartridges may share indispensible role in regulating the function of prefrontal cortex in schizophrenia. The study of postmortem analysis of schizophrenic patient shown decrease in the cartridge cells, which is common in middle cortical layers (80%) than deep and superficial layers [41].

4-Serotonergic neurotransmitter and tryptophan pathway shown some defect based on Lysergic acid diethylamide (LSD) psychosis corresponding with 5HT findings. LSD binds very tightly to the serotonin receptor, causing a greater than normal activation of the receptor. The finding may explain its relation with presence of negative symptoms in Type II schizophrenia, the impairment of memory and the presence of brain degenerative changes. The mechanism based on following evidences: [1] 5HT2 antagonists have an antipsychotic effect of the schizophrenia [2]. The levels of 5-HT2 is decreased in cerebral atrophy in schizophrenia [42]. Furthermore some studies hypothesized that at least two genes, at 22q11, region encoding 5HT2A, are related with schizophrenia [43].

Study findings suggest that the serotonin can antagonize the action of dopaminergic (DE) neuron in the midbrain and the forebrain. This disinhibition of the dopamine in the striatum might moderate the effect of the extra pyramidal system and the prefrontal cortex negative symptoms. Therefore we can use the effect of serotonergic medication to ameliorate the action of dopamine in schizophrenic brain [44]. Olanzapine has a higher affinity for 5-HT_2 serotonin receptors than D_2 dopamine receptors and is used for treatment atypical schizophrenia. The mode of action is unknown, however his high affinity toward 5-HT_2 serotonin receptors might suggest the radical role of these receptors in schizophrenic brain pathogenesis [45]. Correlative studies to test the efficacy of olanzapine as novel antipsychotic drug in the treatment of cognitive impairment in schizophrenia, shown that olanzapine has some superior cognitive benefits relative to haloperidol and risperidone these finding might consul some role of serotonin receptor in the impairment of cognitive process of the prefrontal brain region [46].

5-Noradrenergic pathway and deficit in locus coeruleus might explain some features of schizophrenia, however the studies of noradrenergic marker (dopamine-3-hydroxylase) shown no deficit in schizophrenic brain [12].

The Developmental theory

The new researches hypothesize that multiple histopathology brains changes in the prefrontal cortex, limbic system, ventricles and diencephalon, might happened early in the development of the brain [47]. The structural changes in corpus callosum might reflect the developmental abnormalities in the schizophrenic brain. Magnetic resonance imaging scan (MRI) in the schizophrenic brain shown changes related with reduction in the size of axonal fibers connection in selected area of the brain [48]. Furthermore the is obvious changes in the ventricles including anterior body, isthmus, anterior splenium and changes in the striatal shape [49, 50].

The structural changes seen in schizophrenic brain specially in hippocampal pyramidal cells and the orbitofrontal cortex might related directly with the severity of the symptoms of schizophrenia [51]. The hypothesis of maternal pathogens during the 2nd trimester may interfere with the primitive neuron migration into hippocampus and sequencely effect the pattern of neuronal cell adhesion (N-CAM) maturation due to neuraminidase effect on the cell which might usefully used as a cell marker for schizophrenia [52].

It is well established that there are global changes in the schizophrenic brain including white and gray mater such as: dendrites, synapsis, cell bodies, white mater demyelination, reduce number of oligodendrocytes. This reflects the abnormal myelin conductivity in different regions of the brain as shown by neuroimaging techniques (diffusion tensor imaging). Furthermore, the positron emission tomography using fluorodeoxy glucose reviles decrease glucose metabolism in frontal gray matter and increase metabolic rate in white mater of schizophrenic brain tissue including: corpus callosum, uncinate fasciculus, internal capsule, superior longitudinal fasciculus. This might give a clear idea about the range of the disruptions in the brain function including the connection between frontal and temporal region since myelination of axons is responsible for the rapid transmission of the action potential between different nerve bundles.

The neuroimaging like magnetoencephalography (MEG) resonance also shown the greater loss of the white mater and gray matter volume in the temporal, parietal and frontal region [53]. This might reflect the abnormality in the coding process in these areas and explains the impairment in the memory working process [54]. Schizophrenia is disorder manifests in early adulthood but is thought to be due to disturbances occurring during early brain development. A suggestion of presence schizophrenia gene in the fetal mice brain might unravel the puzzle complexity and help of understanding the pathogenesis of the disease. The study showed that presence of the

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gene that associated with schizophrenia before or after birth (Disrupted-in-Schozophrenia-1) in prefrontal cortex of the mouse shown significant neurodevelopment defects and behavioral abnormalities [55]. There are many evidence indicate the role of glutamatergic neuroreceptor in the cerebral function of the prefrontal region specially at neurodevelopmental stage. The action of NMDA-glutamate receptor antagonist (phencyclidine) and the presence of glutamatergic abnormalities in the schizophrenic brain indicate the relation between this disease and glutamatergic action specially at anterior cingulated cortex and hippocampus [32].

Embryonic stem cells (ES) & production of neuronal cells

Embryonic stem cells (ES) can provide us with numbers of astrocytes, oligodendrocytes and other variant neuronal cells. These (ES) derived from blastocyst of the embryo under the effect of mitogen (signaling molecules). The neuronal cells can differentiated into serotonergic, dopaminergic and other sorts of cells. The analyzing of molecular structure of these cells is a strong tool to understand the mechanism of the psychiatric diseases. Many types of genes (*Pax2, Pax5, Wnt1, En1, Nurr1*) are important for the differentiation of these cells in forebrain, midbrain and hindbrain. For instance two types of OTX homeobox genes are detected (*OTX1* and *OTX2*). *OTX2* is expressed in the undifferentiated stage (stage 1) but decrease gradually in the differentiation stages (stage 2 and 3) while *OTX1* is expressed mainly at stage 3 of differentiation. Tyrosine hydroxylase (TH) is enzyme can used as a marker for ventral midbrain neurons and secreted at stage 4. L-tyrosine is converted to levodopa (L-DOPA) by the enzyme tyrosine hydroxylase. This can be further converted into dopamine, nor epinephrine (noradrenaline) and epinephrine (adrenaline). All these three organic chemicals are known as catecholamines. At stage 5 of differentiation, TuJ1 antibody, can used for diagnosis of differentiated neuronal cells by attachment with Class IIIβ-tubulin. For promoting DA neuron synthesis during stage 4, the cAMP analog, dibutyryl-cAMP, and SHH/FGF8 can add to CNS cultures. The Reverse-phase high performance liquid chromatography (RP-HPLC) can be used to measure dopamine production of neuronal cells [56].

The Demyelination theory and diffusion tensor imaging (DTI)

The new neuroimaging studies of postmortem suggest no evidence of gray matter anomalies, or neuronal loss in schizophrenia. Instead there are many evidence of abnormal connectivity in the white matter between brain regions. The new studies concentrated on oligodendrocytes, which is responsible for myelin formation. They used diffusion tensor imaging (DTI), a type of a magnetic resonance imaging (MRI) technique to study the role of myelin transmission velocity and neural discharges in schizophrenic patient. The electron microscopy has been used to detect the pattern of oligodendrocytes and myelination distribution in a postmortem study of schizophrenia [57].

The study shown presence of 6 sorts of structural abnormalities in schizophrenia comparing with normal brain specimens: In elderly patients the changes in white matter related with negative symptoms while in both young and adult patients the changes more in gray matter and that related with positive symptoms. In addition there is no increase in number of oligodendrocytes in the schizophrenic patient which is normally related with the age. Cuprizone feeding mice model which is used to study of myelin toxicity, shown higher prefrontal cortex dopamine levels with increased CNS activity at early stage. In the late stage both of the demyelination process and loss of oligodendrocyte can occur leading to change in his social behavior, and cognitive deficits. Corresponding with the behavior changes seen in patients with schizophrenia, many deficient mice models have been used as model for demyelination studies such as: Plp1 transgenic mice, NRG1 or its receptor erbB4, Nogo-A [58,59].

Genetics of schizophrenia: animal model

What is a schizophrenic mouse?

In order to study schizophrenia in animal model we need genetic map including all loci related with the disease. Then the mice should be subjected to multiple psychological tests using quantitative trait locus (QTL) to explore the problems of schizophrenia in human beings. The basic in QTL mapping is that the genetic and the phenotypes of the progeny are different from the parental strain by inducing multiple crossing between the parents until production of desirable alleles in the progeny (transgenic strain). For instance parent (e.g., C57BL/6J donor on A/J recurrent parent or A/J donor on C57BL/6J recurrent parent. This system is unique as the progeny

have appropriate chromosome recombination that gives good information related with the genes at molecular level. Animal studies in mice have revealed some of the neural circuitry including medical prefrontal cortex, limbic cortical regions and hippocampus and globus pallidus [59].

Although the genetic study is extensively studied but the result was not conclusive indicating that this type of study is very difficult for multiple reasons: the pattern of schizophrenia distribution has multiple models as the disease is polygenic. The people carrying these genes are not necessary to have clinical features of the disease. Finally, the relation between the phenotype and genes is not simple one, since the transmission not occur according to simple Mendelian law but rather them shown variable expression and low pentrance [60].

The first model of schizophrenic mouse developed in 2007 by John Hopkins researchers. The mouse has both genetic and behavioral changes related with schizophrenia. This model called DISC gene and refer to the shortage of special protein in schizophrenic brain. The mouse is more agitated than normal mouse and cannot swim. Beside that there is also some structural brain changes mimics the changes in schizophrenic brain such as enlargement of the lateral ventricles which can detected easily by using magnetic resonance imaging (MRI). This model may help to denude many genetic as well as the environmental factors that influence the disease process [61].

Chromosomes

Chromosoms6p21.3-22.1, 11q24.2,18q21.2 (neurogranin gene) (NRGN). There are several evidence that support the association of schizophrenia with the immune system of major histocompatibility complex (MHC) region on chromosome 6p21.3-22.1. The first marker located in intron 4 of transcription factor 4 (TCF4) on chromosome 18q21.2. While the other marker located upstream of the neurogranin gene (NRGN) on chromosome 11q24.2. and this might suggest the neurodevelopmental theory of schizophrenia [62].

Chromosome 22 (22q11.2)Df(16)A(+/-) mice study model mouse Df(16)A(+/-) a micro deletion on human chromosome 22 (22q11.2) can contribute to clarify the disconnectivity between frontal and temporal region. The defect in the memory, learning process and its relation to schizophrenia is supported by measurement of prefrontal and hippocampal local field potentials. The study showed decrease performance of the task and reduction in the synchrony of phase-locking of prefrontal cells to hippocampal theta oscillations. In addition, the magnitude of hippocampal-prefrontal action potential during training, learning the task can be measured. Df (16)A(+/-) mice show slow performance and delay task acquisition. These data might support the presence of neuronal disconnectivity in schizophrenia [63].

Chromosome 22q11 (22qDS)

The microdeletions of chromosome 22q11 (22qDS) occur one out of every 4,000 births. It is the second most common anomaly in the human (after trisomy 21) and it occurrence associated with increase the risk of the impairment of intellectual functioning (IQ) in schizophrenia and psychopathology [64].

Chromosome 6q

The dysbindin gene and NOTCH4 on 6p8, 16, COMT, ZDHHC8, XBP1 and PRODH2 on 22q. 10, 15, 20, 21 might increase the risk factor of schizophrenia. There is possibility that these areas are sharing more than one gene and presence of linkage between these genes. Recently this linkage is found on chromosome 6q where five foci have been identified. Chromosome 6q is one of several chromosomal that linked to bipolar disorder (BPD) and schizophrenia (SCZ). Studies also showed that single-nucleotide polymorphisms (SNPs) also found to be related to schizophrenia. For example, linkage to SCZ on chromosome 6p has association with the dysbindin-encoding gene DTNBP1. There is also evidence of sharing other chromosomes (8p and 13q) with NRG1 and G72/DAAO, the dysbindin gene and NOTCH4 on 6p8, 16 and COMT, ZDHHC8, XBP1 and PRODH2 on 22q [65].

Chromosome 22q11.2 deletion

The mice with deletion segment of human chromosome 22,(22q11.2), has a communication breakdown due to less synchrony between two brain area that coordinate learning and memory (hippocampus and the prefrontal region). Correlative evidence in rats divulged that the stimulation of single neurons in the prefrontal cortex firing many neurons in the hippocampus region. The measuring of neural activity in these brain areas can be done by using T-maze memory task. Testing the communication between hippocampus' (produce theta wave) and memory area in striatum region (produce gamma waves) is important for memory working to make the decision (decision-making process) [66].

Chromosome1 (1q21-q22)

Loci in 22 associated with high incidence of schizophrenia has significant evidence of linkage to chromosome 1 (1q21-q22) and might indicate a genetic network actions in schizophrenia [67].

Chromosome 5 (long arm)

The genetic linkage also seen in two DNA polymorphisms on the long arm of chromosome 5 as a responsible of multiple affected members schizophrenia genesis in British and Icelandic families [68].

Chromosome 6(6p24-22)

In Irish pedigrees there is obvious evidence for schizophrenia related with genomic region 6p24–22, *D6S296, D6S285 D6S274* and *D6S296* [69].

Chromosome 15

Nicotinic receptors defect were not previously detected in schizophrenia but according to recent study showed that the relation between decreased function of the α7-nicotinic cholinergic receptor could underlie the physiological defect of schizophrenia. The reduction in the auditory-evoked response (normal inhibition of the P50 to the second stimuli) may related with prefrontal region attention defect in schizophrenia. The decreased P50 inhibition also occurs in some of the patient relatives. This type of inherited susceptibility in schizophrenia is detected in α7-nicotinic receptor of chromosome 15q13-14 [70].

Chromosomes 13q32 and 8p21

Chromosomes 8, 3 and 22, may increase the risk factor of schizophrenia. There is some evidence that the Linkage of chromosome 8 is less than that at chromosome 13, therefore it could be possible that chromosome 8 linkage is false positive linkage [71].

Chromosomes 3p, 5q, 6p, 8p, 20p and 22q

In Finland six chromosomal markers (8p, 20p 3p, 5q, 6p, and 22q) were suggested as candidate genes for schizophrenia, But in the recent study shown no evidence for genetic linkage on these regions [72].

Chromosomes13, 1, 22, 8, 6, 5, 10 (NRG1, catechol-O-methyl transferase, RGS4 and G72 and DTNBP1

Recent advances in relation with heritability of schizophrenia have been a achieved. There is strong evidence of promising genetic regions, for instance, 13q32-34, 6p24-22 and.1q21-22 These 3 regions considered as the most susceptible genetic reaction in schizophrenia. The 22q11-12, 8p21-22, 6q21-25, 1q42, 5q21-q33 and 10p15-p11, are the other promising regions. In addition NRG1, catechol-O-methyl transferase, RGS4 and G72 and DTNBP1 as susceptibility loci, are also promising but not conclusive. Genetic interaction between these genes, possibility of the influence of many environmental factors and presence of epistatic interactions all need to be take in consideration in the recent researches of schizophrenia [73].

Mice genetics models

It is unclear, how genetic liability inducing cognitive deficits , however the use of animal as model with mutated genes can providing help to understand the relation between genetics and clinical features of schizophrenia [74].

Some of these genetic models, molecules and receptors show below [75].

GABA (α(5), γ(2), α(4), δGABA(A), GABA(B1),(GAT1).

a.   Acetylcholine (nAChRβ2, α7, CHRM1).

b.   Dopamine (D1, D2, D3, D4, D5, DAT, COMT, MAO).

c.   Calcium (CaMKII-α, neurogranin, CaMKKβ, CaMKIV).

d.   Dysbindin (DTNBP1).

e.   Neuregulin (NRG1).

f.   Disrupted-in-schizophrenia1 (DISC1).

g.   Reelin.

h.   Proline dehydrogenase (PRODH).

i.   Brain-derived neurotrophic factor (BDNF).

j.   Corticotropin-releasing factor (CRF).

k.   Endocannabinoid systems

cKr mouse model

Many animal models were used to reveal psychopathologies associated with schizophrenia. The direct and simple approach to study of schizophrenia is to deconstruct the brain disease into specific endophenotypes. An endophenotype is a specific anatomical, cognitive biomarker that provides specific information about a specific disease. For instance, psychological test like social withdrawal and pre-pulse inhibition for study schizophrenia. Such endophenotypes can also detected by adding or deleted some relevant genes. Schizophrenia mice have been induced by made some changes in the related genes to study the pathophysiological neuronal connection between different areas in the brain. One of These mutant mice is (*chakragati*, or *ckr*) were induced by microinjection DNA fragment (Ren-2d renin gene into BCF (C57BL/10Rospd X C3H/HeRos) fertilized o¬cyte. The transgenic *ckr* mouse has various biochemicals, anatomical and behavioral deficits and can considered one of the important mice schizophrenic model. Circling behavior is the most important endophenotype in the *ckr* mouse mutant mouse (10 to 80 full body turns per minute). This behavior seen when the mouse is exposed to specific stress or drugs like phencyclidine (N-Methyl-D-Aspartate (NMDA) subtype of the glutamate receptor blocker). This stress (psychotic-like state rotation) can decrease by using antipsychotic drugs like as clozapine and olanzapine. This mouse has also anatomical defect which mimic schizophrenic patient. For instance, the is enlargement of the lateral ventricles, white matter atrophy rather than diffuse brain atrophy. Magnetic resonance imaging (MRI) scans of the lateral cerebral reveal that the enlargement of the lateral ventricle occurs early of postnatal life [76].

COMT gene (Val)

There is evidence for a genetic implicating Val158/108Met polymorphism of the catechol O-methyltransferase (COMT) gene and its relation with increase schizophrenia risk. This relation is strong in European samples while this role is less obvious in Asiatic samples [77].

Cuprizone , Plp1, Nogo-A deficient mice

Cuprizone feeding mice model can be used to study of myelin toxicity. This model showed higher prefrontal cortex dopamine levels and have increased CNS activity at early stage.

In the late stage the demyelination process and loss of oligodendrocyte can occur leading to changes in his social behavior, and cognitive deficits corresponding with the changes behavior seen in patients with schizophrenia, Plp1 transgenic mice, NRG1 or its receptor erbB4, Nogo-A deficient mice models also used for demyelination studies [58].

Df (16)A mice

This models *Df* (16)A(+/-) mice can use to study the pathophysiology of the connectivity between frontal and hippocampus region in schizophrenic brain. The changes can performed through micro deletion on chromosome 22(22q11.2). In one study, the mice model

were subjected to craniotomies under general anesthesia for implantation of two tetrodes in the medial prefrontal cortex and the hippocampus area. The synchronization of neural activity between the hippocampus and the prefrontal cortex has been measured during the performance of a spatial working memory task using T maze task. The task has 2 trails, a 'sample phase', when the animals try to pass into one of two arms, and a 'choice phase', in which the animal should use his memory to know the arm he was visited before. The synchrony of prefrontal-hippocampus area in wild muse was normal while in *Df* (16)A+/− mice the strength of phase-locking was significant lower than in wild mice suggesting reduced hippocampus-prefrontal synchrony in *Df* (16)A+/− mice (decrease theta-frequency synchrony) [78]. These results are consistent with other studies that used T maze task to measured the performance of a spatial working memory task [63].

DISC1 gene

DISC1 mouse is discovered in 2007 and can consider as 1st mouse that has behavioral and physiological defects mimics' schizophrenia. This model can be used for genetic study of the disease. The gene is constructed by truncated of c terminal human DISC1 gene and transfected into C57BL/6 mice by using α calmodulin kinase II promoter. The studies shown that the gene was expressed specially in the granule neurons in the dentate gyrus of the hippocampus, and pyramidal neurons in the prefrontal cortex. The animals mimic schizophrenia anatomically by presence of lateral ventricle enlargement mostly in the left hemisphere and the left hippocampal volume is less than in normal animal. There is also significant decrease in parvalbumin-containing neurons. On the other side, the animal is behaviorally also mimics the schizophrenia in human being: The startle evoked by loud tone (prepluse inhibition) has shown some defect and the muse also showed hyperactivity in open field .The maze test is slower than normal mouse and less performance in forced swim test. The present model might have advantage for study epistatic effect and gene–environment interaction in schizophrenia. [79].

Dysbindin-1protein & *DTNBP1 gene*

Dysbindin-1 is glycoprotein complex found in brain tissue especially in hippocampus but also in sarcolemma of muscle. Its action on presynaptic of glutamatergic neurons suggest that its deficiency in schizophrenia is due to genetic factor related with *DTNBP1* gene that located within chromosome 6p22.3. During neurodevelopment period dysbindin-1 connected with protein β-dystrobrevin at presynaptic region of glutaminergic transmitter. Its presence there in high amount proving the importance of this protein as a risk factor in schizophrenia. Albeit the exact function of this protein is unknown, the recent studies suggest its relation with trafficking process of lysosome in the organelles. Here its worthy to mention that glutamatergic synapse has a complex binding sites of multiple gens such as those encoding G72, D-amino acid oxidase, 1 (*NRG1*), signaling 4 (*RGS4*)., D-amino acid oxidase (*DAAO*), in addition to Dysbindin-1. The later has special effect on VGlutT-1 synthesis, and degradation at glutamate post synaptic region [80].

Recent study (2011) support the relation between dysbindin protein and the effectiveness of specific nerve cell in hippocampus that responsible for very fast brain activity of sound processing. This activity is significantly diminished in mutated mouse as well as in schizophrenic brain. In addition it has been shown that the fast activity of the nerve transmit ion is also important for processing of short term memory in this area of the brain. This evidence may explain the deficit of short memory in schizophrenic patient [81].

Calcineurin protein phosphtase (CN) mice

It is ca^2-calmodulin dependent serine –threonine protein phosphatase and has an important role in T cell function, synaptic plasticity and memory. CN mutants mice have less weight than their wild type and they appear healthy but they have liability to sudden death (mostly with in 6 month age) due to acute health deterioration. CN mutants manifested increase in the locomotors activity more than that of wild strain during an open field test. They also shown abnormal anxiety-like behavior, decreased social interaction, impaired prepulse Inhibition and impaired nesting behavior. CN is important model for immune system study by which CN inhibitors like cyclosporine can be used as immunosuppressant. Beside that there are some side effects of these compounds including delusion, paranoia, depression, agitation, apathy, insomnia and confusion, visual and auditory hallucination. All these symptoms mimic these symptoms found in schizophrenia. In addition, the combination of diabetes with the presence of the immune abnormalities are consistent with the alterations of CN signaling and might reveal the connection between CN, impaired memory function and immune sensitivity in schizophrenia [82].

Neuregulin-1 gene (NRG1)

Neuregulin-1 (NRG1) genes is responsible for regulation function of GABAergic, NMDA receptor, and dopaminergic neurons. Post-mortem studies shown a strong association of increased NRG1 expression with schizophrenic. These studies also shown increased locomotors activity and decrease prepulse inhibition. Beside that there were also increases in a GABAergic marker (parvalbumin) and myelination markers in their frontal cortex. These finding might refer to the relation of NRG1 hyper-signaling with disruption of cognitive and behavioral mechanism in hippocampus and prefrontal region. The animal model studies hypothesize that the down regulation of NRG1 signals in the hippocampus and prefrontal cortex is responsible for the pathophysiology of schizophrenia [83].

It is well established that NRG1 knockout-mutants(ErbB4), has decrease in parvalbumin positive cells in the hippocampus and loss of ErbB4 signaling and that would reduced myelination process by reducing the number of oligodendrocyte. These abnormities are consistent with the findings on postmortem schizophrenic brains. NRG1 has also effect on midbrain dopaminergic neurons by increase the release of dopamine (positive influence on dopaminergic system). However there is negative effect on the syntheses of thyroxine hydroxylase enzyme (HT). This discrepancy might refer to the complexity of neurobiological regulation system [83].

The neuregulin has an important role in proliferation, migration cell survival, differentiation of neurons and glial cell. Immune antibodies against NRG-1 and ErbB4, can be detected in many areas of the human brain including hippocampus, cerebellum, midbrain, the frontal cortex. There are also many brain cells involved in this process like pyramidal neurons, glial cells and white matter of the dorsolateral prefrontal cortex. This wide disturbance in the basic structure of the brain might give evidence of it relation with the schizophrenia [84].

Gene neuregulin 1 (*NRG1*), or (neuregulin) glial growth factor, has multiple SNPs and two microsatellite polymorphisms in Chromosome 8p22-p11. The mutant gene has been found in many schizophrenia patients in the Icelandic and Scottish .These studies shown also a strong positive association with six polymorphisms markers: SNP8NRG221132, SNP8NRG221533, SNP8NRG241930, SNP8NRG243177 and the microsatellite markers 478B14-848, 420M9-1395 [85].

ErbB4 as candidate gene for schizophrenia has great interference with action of pyramidal cells by regulation balance between excitation and inhibition of a major inhibitory neurotransmitter (GABA), especially in prefrontal cortex area. The effect of knocking out the ErbB4 gene in basket and chandelier cells have been studied and shown that both cells have supply GABA to pyramidal cells [86]. The Knockouts mice need longer time to learn and they also spent a lot time sniffing and snooping around. But when these mice have been treated with diazepam, they responded normally. This might explain according to the interference theory of chandelier and basket interneurons in the prefrontal cortex, (inhabation of GABA and supplying it to the pyramidal cells) [87].

NOTCH & Tenascins genes

NOTCH is (transmembrane protein) family has an extracellular domain consisting of multiple epidermal growth factor-like (EGF) repeats, and an intracellular domain. This gene encodes a member of the Notch family including NOTCH1, NOTCH2, NOTCH3, and NOTCH4. The later has a strong relationship with negative symptoms of frontal lobe cognitive performance but only weak association with the volume of frontal lobe. This gene found at region 6p21.3 of the major histocompatibility complex (MHC) and has been proved to be responsible for schizophrenia in Britain. In Chinese population a single nucleotide polymorphism (SNP) at the NOTCH4, rs520692, has been detected. The gene has three microsatellite (TTAT)n repeats in intron 17, the (CTG)n repeats in exon 1 . Tenascins are also a family of extracellular matrix proteins. The gene coding tenascin X (TNXA and TNXB) is located in the MHC class III region. It is located near NOTCH4 locus and they thought to be also important in the brain development [88].

NURR1gene

Nurr1 (NR4A2) is a nuclear receptor and can be considered as one of the important source for dopamine-related problem in schizophrenia. The deletion of Nurr1 show behavioral abnormalities of schizophrenia like increased locomotors activity. The treatment with N-methyl-D-aspartate (NMDA) receptor antagonist, dizocilpine showed significant deficits in the prepulse inhibition test in male but not female Nurr1-deficient mice. However, *Nurr1* deletion did not cause cognitive functions in schizophrenia [89]. *Nurr1* gene

is important for development of dopaminergic neurons in the midbrain of mice. Transgenic Nurr1 gene mice die early because they have deficient in midbrain dopaminergic neurons. This study reflects the importance of brain stem dopamine neurotransmission and its role in the pathogenesis of schizophrenia [90].

OCT-6 (POU) transcription factor

Oct-6, a POU-III domain homeobox transcription factor, are family of genes expressed in the embryonic stem cells in cortical layer 11,111,V. hippocampus and in peripheral nervous system (Schwann cells).It's also expressed in post mitotic neuron of the telencephalon. This study evaluated the expression of Oct-6 in schizophrenia, as psychological disease might associated with neurodevelopment disorders. A special antibody (rabbit polyclonal against oct-6) was used to examine Oct-6 expression in the postmortem brain tissue of schizophrenic patient and others as control group. In situ hybridization, Western blot analysis was used to study Oct-6. The Results was strong Oct-6 immunoreactivity was detected in all brain specimen, especially in dentate gyrus, hippocampus and pyramidal cell layer of frontal region [91]. In contrast with these conclusion, other study showed that the immunoreactivity of Oct-6 expression were observed in schizophrenic patient, non schizophrenic (control group) and in other diseases like bipolar and major depression. There was no difference detected between all these groups [92].

Reelin gene (Reln)

The Mouse reelin gene (Reln) has an extensive disruption in many brain structures. The new evidence supported that Reelin is an extracellular protein direct the neuronal connection process in the developmental period. This gene is present in the human and other vertebrate species, and there is some similarity between mouse reelin and human proteins considering the size and nucleotide sequences. RELN is present in the cerebellum and liver tissue. This gene is part of human chromosome 7q22. Many studies referred to the importance of this gene and its role in the development, function of brain and its relation with various diseases like schizophrenia [93].

Reelin is a secretory protease and is one of the significant molecular findings in schizophrenia. Reduced cortical reelin expression can be used as marker for detection schizophrenia. This reduction has been found in the dorsolateral prefrontal cortex, hippocampal and dentate gyrus. The contribution of reelin in the synaptic pathology of γ-aminobutyric acid (GABA)-ergic neurons might play a major role in neurodevelopment and plasticity [40].

Both of reelin and glutamic acid decarboxylase (GAD) 67 are deficient in reeler mice, the model mouse of schizophrenia. They effect the production of gamma-aminobutyric acid neurotransmitter in brain and both of them are decrease in schizophrenic patient. Some studies suggest that this reduction might occur through epigenetic mechanisms (i.e., cytosine hypermethylation of CpG islands in the gene promoter). The treatment with protracted l-methionine would increase of S-adenosyl-homocysteine, hence the reduction of reelin and GAD67 mRNAs can be detected in reeler mice. This effect of l-methionine was responsible for increasing the number of methylated cytosines in the reelin promoter CpG Island [94]. The mutant reeler mouse, shown decreased in both of GABAergic neurotransmitters and reelin expression in the prefrontal area of the brain. Hence these mice model for schizophrenia shown deficit in cognitive, learning process and visual attention [95].

RGS4

G-protein signaling 4 (RGS4) is significantly diminished in the schizophrenic prefrontal cortex. cDNA microarrays quantitative in situ hybridization has been used to confirm the result of the microarray manifested decreases in RGS4 expression in three schizophrenic cortical areas of the brain. The microarray study of 70 genes related with schizophrenia at locus 1q21--22 shown that RGS4 gene expression was consistently present in all samples, indicating that the RGS4 depletion expression mainly due to effect of neuronal signaling changes [96].

Bdr/+ mice model (Disrupted circadian rhythms in a mouse model of schizophrenia)

Bdr/+ mice; protein (SNAP)-25-encoding gene blind-drunk show sleep-wake disturbances that mimic schizophrenic patient by change light - dark cycle pattern. This disturbance is not related to disturbance of the light input pathway nor defect in suprachiasmatic

nuclei (SCN) which represent the central mechanism of rhythms in light-dark cycles regulation. There is instead some evidence of neurotensin (Nts) and argentine vasopressin (Avp) changes. The Bdr/+ as mutagenic mouse can provide a good evidence of rhythmic dark-light disturbance in the patient of schizophrenia [97].

(DAT knockout mice) Reduced tumor growth in a mouse model of schizophrenia, lacking the dopamine transporter

A new study supports a hypothesis that Dopamine (DA), interferes with the signaling process in endothelial cells, blocks its angiogenic functions and inhibits tumor growth. According to this theory, the incidence of the tumors in schizophrenic patient has been reported to be less than the incidence in other people. The investigation of the level of tumor growth and angiogenesis in the mouse model of schizophrenia that lacking the dopamine transporter (DAT knockout mice) support this hypothesis [98].

However, this is not the case for other sympathetic neurotransmitters (catecholamine) such norepinephrine (NE) and epinephrine (E). Both have stimulating affect on tumor vascularization (angiogenic function) and then they enhance the growth of cancer cell. These guessed that the modulation of the dopaminergic system may contribute to medication treatment of schizophrenia (anti dopaminergic antipsychotic drugs) as well as cancer therapy [99].

RGS4, DISC1, DTNBP1, STX7, TAAR6, PPP3CC, NRG1, DRD2, HTR2A,DAOA, AKT1, CHRNA7, COMT, and ARVCF genes

In one of the largest study including more than 1,800 schizophrenic case to show the relation of the common single nucleotide polymorphism(SNP) with schizophrenia concluded that: no one of the candidate genes(*RGS4, DISC1,DTNBP1, STX7, TAAR6, PPP3CC, NRG1,DRD2, HTR2A, DAOA, AKT1, CHRNA7,COMT,* and *ARVCF*) that selected has significant effect as predisposing factor for schizophrenia [100].

A proposal study project

Method and materials

1-Animals robust genes

Currently, there are many mutant genes used to study schizophrenia but the most susceptible genes are these encoding neuregulin (*NRG1*) and dysbindin (*DTNBP1*). The studies data are promising, but not definite for other genes such as: regulator of G-protein signaling 4 (*RGS4*), disrupted in schizophrenia (DISC1), V-AKT murine thymoma viral oncogene homolog 1 (*AKT1*) and D-amino acid oxidase activator (DAOA) [8].

Neuregulin-1 gene (NRG1) study is our first choice

We prefer to choose Neuregulin-1 (NRG1) mice model for the following reasons:

A. Neuregulin-1 (NRG1) genes are responsible for regulation function of 3 receptors in the brain: GABAergic, NMDA receptor, and dopaminergic neurons. Postmortem studies shown a significant association of increased NRG1 expression with schizophrenia.

B. These studies also have shown increased locomotors activity, decrease prepulse inhibition. Beside that there were also increases in a GABAergic marker (parvalbumin), myelination markers in their frontal cortex. These finding might refer to the relation of NRG1 hyper-signaling with disruption of cognitive band behavioral mechanism in hippocampus and prefrontal region [101].

C. NRG-1 or its receptor ErbB4has an important role in proliferation, migration cell survival, and differentiation of neurons and glial cells. This wide disturbance in the basic structure of the brain might give evidence of its strong relation with the schizophrenia [84].

ErbB4 as candidate gene for schizophrenia has great interference with action of pyramidal cells [81]. On the other hand dysbindin (*DTNBP1*) is also robust model but its action restricted on presynaptic of glutamatergic neurons [80]. We intend to study 300 mice model Neuregulin-1 (NRG1) of both sex (150 males &150 females). All mice should be subjected to psychological tests and MRI before and after the operation (Stereotactic deep brain stimulation). The psychological test will include both of Prepulse inhibition test (PPI) and (Wisconsin Card Sorting Test). Startle test measures the sensory gating in the P50 event-related potential). It is one of the most

robust tests with high validity and does not need learning. This test depends on the presence of weak pre stimulus before startle amplitude reduction. Wisconsin Card Sorting Test is relevant to the cognitive deficits associated with schizophrenia. We can detect the social behavior and cognitive impairment of working memory and attention [102].

Only 150 of these schizophrenic (NRG1) models (75 females & 75 males) will be subjected to stereotactic operation targeted nucleus accumbens by using beep brain stimulation (DBS). The selection of these 150 animals should be randomly (double blind procedure). All experiment should be carried out with approval of the ethical committee at Utrecht University.

The psychological tests and MRI tests would be repeated after 7 days (The period is suitable for complete recovery). The animals that would show any post operative complications like wound infection or fever can be detected clinically while the using of MRI would be of a great help to detect brain abscess, cerebritis and intracerebral hemorrhage. All these cases should be excluded from doing the post operative tests. We prefer to start with MRI test to follow the anatomical lesion in Nucleus accumbens before starting with our postoperative psychological test. The changes on the behavior and memory of the animals can be detected to compare the results of the tests (pre & post operative) and then to be followed up post operatively.

2-Investigations in schizophrenia

Psychological tests in animal models

Animal models have been used to mimic schizophrenia disorders in the human; however it's difficult to find all symptoms of schizophrenia in the animal model. Hence schizophrenia is complex clinical disorder and reflects many factors (genetic as well as environmental) and it is impossible to induce all these changes in one model. Therefore we have to focus on specific symptoms associated with schizophrenia, rather than the entire clinical features of the disease. Another problem is to choose which test is valid to study schizophrenia in animal model. Some of these tests are related with memory work, selective attention, sensor motor gating and preservation. In behavioral measures we can also used impaired attention test or disruption of prepulse inhibition test. Both of them are mimic these test used to diagnose schizophrenia in human and are probably valid tests [103].

However some of abnormalities such as delusions and hallucinations have genetic backgrounds and we can evaluate these symptoms better depending on the study of cellular and molecular component in schizophrenic genetic model or according to developmental theory. For instance the study of locomotors activity in schizophrenic mice model depend largely on dopamine hypothesis. *N*-methyl-D-aspartate (NMDA) antagonists, such as phencyclidine (PCP) can induce hyper activity and behavioral changes mimic schizophrenia [102].

The response reduction during repetition of same unimportant stimuli in the absence of emergency state is called habituation tests. Prepulse inhibition test (PPI), startle test can be used to measure the sensory gating in the P50 event-related potential). This test depends on the presence of weak pre stimulus before startle amplitude reduction. It is one of the most robust tests with high validity and does not need learning. During P50 Gating test, the 2 rapid acoustic clicks are presented with period of 500 milliseconds between them. In normal persons, the P50 event (potential of the 2nd click) is decrease in relation with the 1st click. This response would decrease (less gating) in schizophrenic patients.

We can also detect Social Behavior and Cognitive impairment of working memory and attention (Wisconsin Card Sorting Test). During visual discrimination test (black vs. white maze) arm, we need first to learn the mouse to obtain a food reward. This test is relevant to the cognitive deficits associated with schizophrenia [97].

MRI findings in schizophrenia (Human and animal model)

There are many abnormality detected in post mortem schizophrenic brain by using MRI. Some of these abnormalities are large and obvious including: The frontal lobe abnormalities (orbitofrontal regions, prefrontal gray matter), temporal lobe structures (superior temporal gyrus, amygdala and parahippocampal gyrus, hippocampus gyrus and neocortical temporal lobe), parietal lobe abnormalities (inferior parietal lobule, angular gyri, supramarginal) and subcortical abnormalities (i.e. basal ganglia, cavum septi pellucidi, corpus

callosum, and thalamus). There is also some evidence for cerebellar involvement. But the most important abnormalities are: lateral and third ventricle enlargement. Other abnormalities are subtle including both grey and white mater in different parts of the brain. The presence of such extensive brain abnormalities may suggest the neurodevelopmental origin, although the role of other factors such as: environmental factors, neurotoxicity, drugs, psychological stress, presence of other disease like diabetes mellitus, might play a role in the occurrence of the disease in addition to early neurodegenerative changes [104].

Smooth pursuit & Antisaccade eye movement (human only)

The antisaccades normal rapid eye movement in the opposite direction when a sudden movement of visual target occur. Anti saccade performance is associated with increase error rate, can be found in schizophrenia (Impaired smooth pursuit eye movement) (SPEM) by 50–80% in schizophrenia patients and 30–40% of their siblings, while it is only 8% of healthy individual [105].

Antipsychotic medication experiments shown that, treatment with risperidone can improve antisaccade errors, via its effect to modify the neuronal disconnectivity in pre frontal region and coulomotor cranial nerve [106]. The clinically unaffected siblings of schizophrenia patients shown intermediate, antisaccade movement (intermediate error between the schizophrenic patient and the controls) characterized by reduced antisaccade gain. This might indicate that the deficit of SPEM and antisaccade can also occur in healthy sibling of schizophrenic patient. Therefore the SPEM deficit can be used as markers (endophenotype) in detection of liable persons for schizophrenia.

The cerebral blood flow (rCBF) (human and animal model)

The cerebral blood flow (rCBF) in schizophrenic patients shown specific pattern of perfusion which decrease specially in medial temporal lobe, pre frontal, sub cortical nuclei and limbic system. This disturbance of blood flow in related brain area and the perfusion poverty might be the main cause of the function disturbance in the neural network [107].

In relevant study three types of tests have been performed during measurement of regional cerebral blood flow (rCBF) in schizophrenic patients and control group to evaluate the performance of the dorsolateral prefrontal cortex (DLPFC) in both groups. The (rCBF) test using xenon (Xe 133) inhalation shown: During rest, the patients had significantly (rCBF) reduction while during NM, no change has been detected comparing with control group. However during Wisconsin Card Sort (WCS), which is specific cognitive test related to DLPFC- area of the brain, schizophrenic patient did not shown any increase in DLPFC rCBF, in contrast to the normal (control group) which showed a significant increase in DLPFC rCBF. The changes that occur during Wisconsin Card Sort (WCS) performance in the normal persons are specific and related to DLPFC area only. This explains why the schizophrenic patient did not shown any increase in DLPFC rCBF in this area during (WCS) test performance [108].

Deep brain stimulation (human and animal model)

DBS is a reversible and adjustable neuropsychiatry procedure, used for investigation and treatment of multiple intractable neuropsychiatric disorders and can be used in combined with stereotactic frame to induce précis functional lesion in brain [109]. A number of techniques are used to change the electrical activity of the brain such as electroconvulsive therapy, a temporary current is delivered to the brain across the scalp and skull. Transcranial magnetic stimulation act by using special electromagnetic coil placed on the scalp to induce currents in brain.

Deep brain stimulation (DBS) act by using a stereo tactically implanted electrodes into specific brain targets. The lead (electrodes), which is 1.27 mm in diameter, is contact on four platinum/iridium electrodes. There are usually 2 leads; each has been connected via an extension wire to pulse generators. The intensity, frequency and pulse amplitude should be regulated. The usual program of stimulation include: a voltage range of 0-10.5 volts, frequency of 2-185 Hz and pulse (60-450) μsecs.

Magnetic resonance imaging and computed tomography are used for anatomic localization. During the implantation of stereotactic application, stereotactic brain atlases is important to determine the précis target of Subcortical nuclei (thalamic nuclei, globus pallidus interna, and subthalamic nucleus). DBS can used for treatment of different neurologic disorders' like pain, dystonia, epilepsy,

brain injury, intractable obsessive-compulsive disorder, parkinsonism and bipolar depression but treatment of positive symptoms in schizophrenia is relatively new and rare. The results of DBS are variable according to the type and severity of the case. For instance, an improvement of 40-70% has been recorded in motor activity in Parkinson's disease by the induction of DBS in globus pallidus and subthalamic nucleus. The initial DBS results in treatment of positive sign of schizophrenia are promising but the efficacy of these data are still uncertain [110].

Deep brain stimulation might induce functional lesion in the stimulation target, through current action potential on neuronal fibers. Although the mechanisms of action are not fully elucidated, it has been suggested that it might modulate the increasing dopamine levels in the striatum by inducing change in the metabolic activity in prefrontal, hippocampus and its connection with limbic basal ganglia. The other possibility of mechanism is by stochastic resonance (reducing chaotic information processing). It has been hypothesized that the electrical stimulation of the hippocampus or nucleus accumbens can stabilize, correct or inhibit of neural activity at these region. It has been proposed to inhibit transmission via one mechanisms: synaptic fatigue, depolarization blockade or "neural jamming" through producing therapeutic functional lesion [110].

It was convincingly shown that the mechanism of DBS is combination of 3 mechanisms: inhibition of GABAergic afferents , depolarization blockade of the neurons (change of on the cell membrane voltage-dependent ion channels) and synaptic depression by orthodromic efferent axons stimulation which lead to transmission inhibition (exhaustion of the neurotransmitter pool) [111].

They are many advantages of using DBS: The procedure is relatively safe, hence only rare complications related with procedure have been detected like seizure, hemorrhage and infection. Others including dysarthria, and diplopia, paresthesias and muscle contraction have been also detected. The functional lesion of DBS is reversible comparing with conventional ablative neurosurgery. In addition, all possible consequences of lead implantation and the stimulation can be modified [112]. Schizophrenia is differ from other psychiatric diseases by which Multiple targets may need o treat all symptoms of the disease. The stimulation of one target, for instance DBS in dopamine target area may lead to decrease the positive symptoms only but not the negative symptoms (cognitive symptoms). Some of hypothetical studies suggest use of DBS in the nucleus accumbens or hippocampus to treat refractory schizophrenia [113].
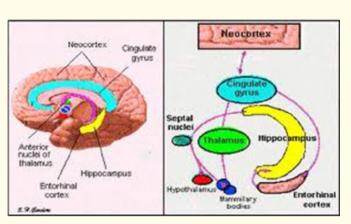
3-Neurosurgical interventions in schizophrenia

The Psychosurgical procedures of intractable mental diseases including schizophrenia and other neuropsychiatric illness like anxiety, aggression obsessive-compulsive disorder, , depression, has shown some improvement in surgical techniques, with aid of neuroimaging techniques such MRI, fMRI and stereotactic technical method [114].

Selection of the lesion sites

The selection of lesion sites depend largely on Papez circuit (see Figure 1), which hypothesized that the neuronal fibers of emotion have circular fashion structure started from cingulum bundle passing to the hippocampus, fornix and then to hypothalamus and thalamus to return back to cingulated gyrus. Schizophrenia has hypo and hyper brain functionality. In order to interfere surgically with schizophrenia we should think not only of destructive lesion in dopaminergic area to control the hyper -functionality but also should enhance the function of hypoactivity area by local electrical stimulation, cell transplantation, genetically modified vectors, or Implantation of mini pumps [115].

The first evidence of beginning of psychosurgery in 1930, when Carlyle Jacobsen had the first frontal loboctomy on Chimpanzee to study the behavioral changes in animals. Stereotactic neuropsychosurgery started at 1960 including caudate nucleus, subcaudate tractotomy, substantia innominata (beneath the head of each caudate nucleus), limbic leucotomy, cingulate areas and anterior capsulotomy [116].

Figure 1: *Papezcircuit. Shown AT, anteriorthalamusA, amyg- dala;;;H,hypo-thalamus; M,mammillary body; S,septalarea. CC,corpuscallosum.*

Indications of stereotactic neurosurgery (human)

The major indications for stereotactic neurosurgery are treatment of refractory obsessive compulsive neurosis and refractory major depressive disorder [117]. The results of neurosurgery in schizophrenia are variable and rare. Intractable chronic schizophrenic patients were treated by induce multiple nuclei lesions under guide of stereotactic CT scan. More than 2/3 of the patient shown significant improvement with a few complications [118].

There is evidence of improvement in functioning of multiple patients with intractable chronic schizophrenia after stereotactic cingulotomy. These improvements included a decrease of suicidal risk, reduction of anxiety and violent behavior [119]. recently more than one thousand patient with different psychotic disorder including chronic schizophrenia were subjected to (DBS) by inducing brain lesions targeting in many brain foci, shown that schizophrenia had the least improvement [120]. In another study, 180 patient suffering from chronic schizophrenia, all subjected to stereotactic treatment by inducing multiple lesions shown obvious improvement of more than ½ of the patient [121].

Destroying bilateral substantia innominata in order to induce thalamo- frontal tractotomy for treatment of different psychiatric illnesses was started in 1961 by using stereotactic operation by inducing a bar hole just above the orbital roof and implantation of radioactive Ytrrium Y90 seeds . The patients shown obvious improvement [122]. Recently the induction of stereotactic lesions into fronto-cingulate fibreor in fronto-thalamic has many advantages comparing with such crude operation of frontal lobe that have been done during the last 60 years [123]. Stereotactic electrical stimulation of nucleus accumbens or the hippocampus has been used to prevent the excessive dopamine release and to stabilize the neuronal network action potential .This operation might be useful for treatment the positive symptom of schizophrenia [118].

The operative procedure (animal model)

The important issue during the procedure of magnetic resonance imaging (4.7 Tesla MRI scanner) or the more advanced MRI, (Murine MRI at 9.4T), is that the candidate mouse head must fixed with a stereotactic device to reduce the mobility during anesthesia and to create high image resolution. But there is an addition problem of hypothermia or death due to decrease the body temperature of the animal under anesthesia. Here we need special device to keep on normal temperature (37°C). There are three options to preserve the heat: Using fluid piping, air piping or fluid piping device. These measurements are very important concerning the safety and research ethics [124].
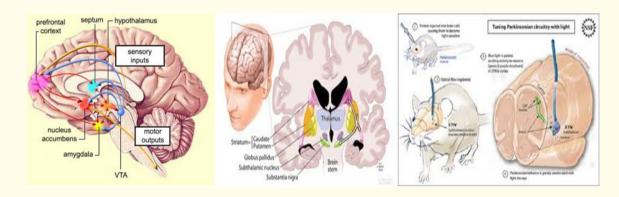
The special MRI used for the mouse (4.7 Tesla MRI scanners) contain special coil to befit with circular cradle of 36 mm. This would maintain the requirement of high resolution of brain images. Beside that the MRI is correlated with special stereotactic device that

compatible with the head size of the mouse .The device should not disturb the magnetic field by using any metallic piece. The animal head position should be central located and should be fixed correctly by stereotactic device. The special anesthetic tube and mask ought to fit the mouse mouth without obstruction or inducing disturbance by presence of MRI device. The special stereotactic device (David KOPF instruments Model 325: MRI Head Holder for Mouse) has used 3 securing sites (two ears screws and one tooth hook) [125].

The targets of DBS

Many evidence suggest that hippocampus through its connections with the limbic basal ganglia, is responsible for dopamine release. Therefore we hypothesized that the DBS of nucleus accumbens (depicted in figure 2) or the hippocampus might lead to decrease the hallucination, illusion and other positive symptoms of schizophrenia.

There are 2 important midbrain pathways responsible for secretion of dopamine these are: nigrostriatal system and the mesolimbic system. The nigrostriatal system is connected to substanti anigra, while mesolimbic system connected with ventral tegmental area (VTA), substantia negra and caudate nucleus. It seems to be that the mesolimbic pathway is mostly responsible for schizophrenic positive signs. There are two different modes of dopamine release in midbrain (The tonic/phasic hypothesis of DA release): The Phasic activation lead to increase the level of DA abruptly and it thought to be important for reward basis learning process (incentive learning). The tonic activation on the other hand leads to stable release of DA. The abnormal excessive release of DA in phasic activation via abnormal hyperactive hippocampus in schizophrenia is thought to be more important to induce the positive symptom. In addition, the activation of Nucleus accumbens (GABAergic neurons) by DBS can inhibit ventral tegmental areas (VTA dopaminergic neurons) which ultimately lead to decrease of DA release and decrease the positive symptoms in schizophrenia. For these 2 reasons, we propose that DBS should be preceded in one of these areas or both of them. Alternatively the other targets such as ventral pallidum nucleus (VP) and ventral tegmental area (VTA) can be also two candidates for DBS [109].

Figure 2: *The left side figure shown :Nucleus accumbensin the human brain-The brain main nucleus responsible for drugs addiction reward and motivation (incentive motivation). . The middle figure refer to the position of deep brain nuclei in the human brain, while theright side figure represent the position of the deep brain nuclei in the muse.*
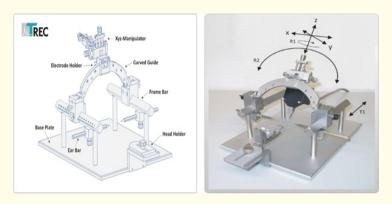
The stereotactic operation techniques

The stereotactic operation techniques are the same for all operations followed by inducing drill hole in the cranium of muse. Such operation is used for various purposes like excision of brain glioma, geneo-therapeutic, xenograft implantation. [126]. The system consists of guide of a 2.6 mm that accept Hamilton syringe needle of 26-gague of 0.5 mm diameter. The procedure started after fixing the animal cranium with stereotactic screws (Kopf Instruments, Sunland, CA) one for frontal teeth and other two screws on each side of temporal bone.

We can use a mixture of Ketamine and Xylazine to be inducted by using intra-peritoneal injection .The general anesthesia induction is according to the standard protocol [127]. During induction of anesthesia more cushion must be directed toward observation of

the body temperature to prevent hypothermia and death of the animals. The operation should be done under sterile condition using alcohol with iodine solution for washing and disinfected the site of operation to avoid the infection of skin and brain tissue. Small skin incision (1 cm midline scalp incision) induces at the frontal bone, followed by induction of a bar whole into frontal bone with aid of 33 G drill, in the distance of 0.5 mm anterior, 2 mm lateral to the bregma [127].

We can also use screw implantation with guide system (33-gauge Plastic cannula) to be inserted at 3.3 mm depth to frontal bone, and then to be directed toward the nucleus accumbens under the direct MRI guidance [121]. The needle is removed from the guide and substituted with the high frequency electric electrode .The intensity, duration amplitude of the frequency current of DBS in the mouse brain is induce according to the instruction of stimulation protocol. The bar hole to be occluded with bone wax and the incision closed using wound glue. Psychological test and cognitive observations tests will be performed 7 day after operative recovery'.

Figure 3: (left figure), The small animal stereotactic instrument(SASI), an easy to be used instrument in small animals stereotaxic ,there is enough place for placement of cannula ,electrodes, and other devices. right Figure SASI has good degrees of freedom including: mouse adaptor , Head holders ,XYZ-Manipulator X,Y, Z adjustment, Rotation adjustment, and angle adjustment.

Stimulation protocols

DBS using a magnetic stimulator MagPro R100 (MagVenture, Skovlunde, Denmark, 60-75 A/μs), has a circular coil (Type MC-125; diameter = 125 mm, thickness = 11.3 mm). The DBS program in human consisted of 100 μs pulses at 130 Hz for 30 s, with 30 s of base-line activity before and after high frequency stimulation (HFS) [129]. While in the mice, the intensity of the magnetic stimulus should be adjusted to 60–75 A/μs and the program used is: 10 trains of 20 pulses at 100 Hz with 1s intervals [130].

The operative complications

Are relatively rare including acute complications such as Hemorrhage and infection are the most important acute complications. Hemiplegic, epilepsy, transient confusion, lethargy, incontinence of urine and transient postoperative brain edema also can occur.

While the longer-term complications include: epilepsy, irritability, lack of consideration and lack of initiative state [131].

Summary

Schizophrenia is mental illness, characterized by presence of both positive and negative symptoms. The recent antipsychotic medications are failed to achieve a good result in about 30% of the cases and this can lead to increase the numbers of the suicidal attempt. In addition these medicines have many intolerable side effects. In order to achieve suitable solution for treatment the disease, we assume that the DBS in specific deep nuclei of the brain would achieve a good effect regarding the modulation of the disturbance in the neuronal network.

Nucleus accumbens (NAc) is one of the important centers for dopamine regulation. In addition, the activation of Nucleus accumbens (GABAergic neurons) by DBS can inhibit ventral tegmental areas (VTA. dopaminergic neurons) which ultimately lead to decrease of DA release and decrease the positive symptoms in schizophrenia.

We hypothesize that the DBS would have good results regarding the positive symptoms of disease depending on the basis of dopamine hyperactivity theory of schizophrenia. A number of authors are emphasis upon the importance of the genes encoding neuregulin (NRG1) as candidate for study schizophrenia in mice model.

These models would be subjected to DBS under control of MRI. The important issue during the procedure of magnetic resonance imaging (4.7 Tesla MRI scanner) or the more advanced MRI, (Murine MRI at 9.4T), is that the candidate mouse head must be fixed with a stereotactic device to reduce the mobility during anesthesia to create high image resolution. The small animal stereotactic instrument (SASI) would be used in our model since the SASI is a easy instrument to be used in mice models and there is enough place for placement of cannula, electrodes and other devices.

The stereotactic operation techniques would be followed by inducing drill hole in the cranium of muse frontal bone in the distance of 0.5 mm anterior, 2 mm lateral to the bregma with aid of 33G drill.

The intensity of the magnetic stimulus should be adjusted to 60–75 A/µs and the program would be: 10 trains of 20 pulses at 100 Hz with 1s intervals. On the 7th post operative day the animals would be again subjected to psychological behavioral tests to compare between the pre and post operative results concerning the effect of the DBS on the positive symptoms.

We suggest that the use of the prepulse inhibition test (PPI) in mice can mimic the test used to diagnose schizophrenia in human and is probably valid test. It is one of the most robust tests with high validity and does not need learning. We can also detect Social Behavior and Cognitive impairment of working memory and attention (Wisconsin Card Sorting Test).

The Hypothesis Evaluation

We can convincingly evaluate the hypothesis by following up our models at pre and post operative period using the aid of fMRI or positron emission tomography (PET) to observe the changes in the brain metabolic. We can also use Biomolecular assay including the dopamine concentration level in the cerebrospinal fluid (CSF) [132].

Bibliography

1. Elkis H., *et al.* "Refractory schizophrenia". *Revista Brasileira de Psiquiatria Suppl* 2 (2007): S41-S47.
2. Ellenbroek Bart., *et al.* "Can 5-HT3 antagonists contribute toward the treatment of schizophrenia". *Behavioural Pharmacology* 26.1-2 (2015): 33-44.
3. G Remington., *et al.* "Conventional versus novel antipsychotics: changing concepts and clinical implications". *Journal of Psychiatry and Neuroscience* 24.5 (1999): 431-441.
4. Candan Depboylu., *et al.* "Systemically administered neuregulin-1β1 rescues nigral dopaminergic neurons via the ErbB4 receptor tyrosine kinase in MPTP mouse models of Parkinson disease". *Journal of neurochemistry* 133.4 (2015): 13020.
5. Norton Nadine., *et al.* "An update on the genetics of schizophrenia". *Current Opinion in Psychiatry* 19.2 (2006): 158-164 .
6. Udani Ratnayake., *et al.* "Dopaminergic activity and behaviour in SOCS2 transgenic mice: Revealing a potential drug target for schizophrenia". *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 56.2 (2015): 247-253.
7. Timothy J Spellman., *et al.* "Synchrony in schizophrenia: a window into circuit-level pathophysiology". *Current Opinion in Neurobiology* 30 (2015): 17-23.
8. Mesbah Alam., *et al.* "Neuronal activity of the prefrontal cortex is reduced in rats selectively bred for deficient sensorimotor gating". *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 56.2 (2015): 174-184.
9. SahaS., *et al.* "A Systematic Review of the Prevalence of Schizophrenia". *PLoSMedicine* 2.5 (2005): e141.

10. Faris R E L., *et al*. "Mental disorders in urban areas: an ecological study of schizophrenia and other psychoses". *American Journal of Public Health* 50.9 (1960): 1455.

11. Samuel J and Keith MD "Pharmacologic Advances in the Treatment of Schizophrenia". *New England Journal of Medicine* 337 (1997): 851-852.

12. T J Crowr. "Molecular pathology of schizophrenia: more than one disease process?" *British Medical Journal* 280.6207 (1980): 66-68.

13. White L., *et al*. "Empirical Assessment of the Factorial Structure of Clinical Symptoms in Schizophrenia". *Psychopathology* 30.5 (1997): 263-274.

14. Goldman-Rakic and Patricia S. "Working memory dysfunction in schizophrenia". *The Journal of Neuropsychiatry and Clinical Neurosciences* 6.4 (1994): 348-357.

15. David A Lewis and Guillermo Gonzalez-Burgos. "Pathophysiologically based treatment interventions in schizophrenia". *Nature Medicine* 12.9 (2006): 1016-1022.

16. EC Johnstone., *et al*. "cerebral ventricular size and cognitive impairment in chronic schizophrenia". *Lancet* 2.7992 (1976): 924-926.

17. McGlashanH and Thomas. "Classical Subtypes for Schizophrenia. Literature Review for DSM-IV". *Schizophrenia Bulletin* 17.4 (1991): 609-632.

18. Torgersen Svenn., *et al*. "Relationship of Schizotypal Personality Disorder to Schizophrenia: Genetics". *Schizophrenia Bulletin* 11.4 (1985): 554-563.

19. P J Harrison., *et al*. "Schizophrenia genes, gene expression, and neuropathology: on the matter of their convergence". *Molecular Psychiatry* 10.1 (2005): 40-68.

20. Snyder Solomon H. "Amphetamine psychosis: A "model" schizophrenia mediated by catecholamines". *The American Journal of Psychiatry* 130.1 (1973): 61-67.

21. A Gray., *et al*. "The neuropsychology of schizophrenia. Department of Psychology, Institute of Psychiatry". *Dialogues in Clinicale Neuroscience* 12.3 (2010): 383-392.

22. Otte DM., *et al*. "N-acetyl Cysteine Treatment Rescues Cognitive Deficits Induced by Mitochondrial Dysfunction in G72/G30 Transgenic Mice". *Neuropsychopharmacology* 36.11 (2011): 2233-2243.

23. Victoria Campos-Peña., *et al*. "The Kynurenine Pathway at the Interface Between Neuroinflammation, Oxidative Stress, and Neurochemical Disturbances: Emphasis in Schizophrenia". *Studies on Psychiatric Disorders* (2014): 245-268.

24. R Diaz Heijtz., *et al*. "Normal gut microbiota modulates brain development and behavior". *Proceeding of the National Acadamy of Science of the USA* 108.7 (2011):3047-3052.

25. CT Jones., *et al*. "Mutation in codon 713 of the amyloid precursor protein gene presenting with schizophrenia". *Nature Genetics* 1.4 (1992): 306-309.

26. Knight JG "Is schizophrenia an autoimmune disease? A review". *Methods and Findings in Experimental and Clinical Pharmacology* 6.7 (1984): 395-403.

27. Paul H. "Biology Division, California Institute of Technology, Pasadena, CA 91125 USA.

28. Sarnoff A., *et al*. "Adult Schizophrenia Following Prenatal Exposure to an Influenza Epidemic". *Archives of General Psychiatry* 45.2 (1988): 189-192.

29. Ravit Hadar., *et al*. "Using a maternal immune stimulation model of schizophrenia to study behavioral and neurobiological alterations over the developmental course". *schizophrenia Research* 166.1-3 (2015): 238-247.

30. Takahiro Tsujita "Genomic Discordance Between Monozygotic Twins Discordant for Schizophrenia". *American Journal of Psychiatry* 155.3 (1998): 422-424.

31. Fujii Yo., *et al*. "Positive associations of polymorphisms in the metabotropic glutamate receptor type 3 gene (GRM3) with schizophrenia". *Psychiatric Genetics* 13.2 (2003): 71-76.

32. Tamminga CA., *et al*. "Schizophrenia and glutamatergic transmission". *Critical Reviews in Neurobiology* 12.1-2 (1998): 21-36.

33. Khalid Mansour., *et al*. "The Glutamate theory vs the Dopamine theory in schizophrenia . (III) Glutamate Linked Treatments of Schizophrenia" (2012).

34. RG Chris Turck., *et al*. "Characterization of molecular pathways in schizophrenia et Institute of Human Genetics, University of Bonn ,Germen research institute of psycology.

35. Davis Kenneth., *et al*. "Dopamine in schizophrenia: A review and reconceptualization". *The American Journal of Psychiatry* 148.11 (1991): 1474-1486.

36. Stephen J., *et al*. "Uneven Pattern of Dopamine Loss in the Striatum of Patients with Idiopathic Parkinson's Disease". *New England Journal of Medicine* 318.14 (1988): 876-880.

37. Philip Seeman., *et al*. "Dopamine D4 receptors elevated in schizophrenia Pharmacology and Psychiatry Departments". *American Journal of Medical Genetics* 54.4 (1994): 384-390.

38. David L., *et al*. "Sensorimotor Gating and Schizophrenia Human and Animal Model Studies Department of Psychiatry". *Archives of General Psychiatry* 47.2 (1990): 181-188.

39. Kyungheup Ahn., *et al*. "Probing GABA Receptor Function in Schizophrenia with Iomazenil". *Neuropsychopharmacology* 36.3 (2011): 677–683.

40. Sharon L Eastwood., *et al*. "Cellular Basis of Reduced Cortical Reelin Expression in Schizophrenia". *The American Journal of Psychiatry* 163.3 (2006): 540-542.

41. Joseph N., *et al*. "Alterations in Chandelier Neuron Axon Terminals in the Prefrontal Cortex of Schizophrenic Subjects". *The American Journal of Psychiatry* 156.11 (1999): 1709-1719.

42. AvrahamBleich., *et al*. "The role serotonin in schizophrenia". *Schizophrenic bulletin* 14.2 (1988): 297-315.

43. McDonald C, Murphy KC: The new genetics of schizophrenia.Division of Psychological Medicine, Institute of Psychiatry, de Crespigny Park, London SE5 8AF, United Kingdom.

44. Kapur Shitij., *et al*. "Serotonin-dopamine interaction and its relevance to schizophrenia". *The American Journal of Psychiatry* 153.4 (1996): 466-476.

45. Ellingrod VL., *et al*. "5-HT2A receptor promoter polymorphism, -1438G/A and negative symptom response to olanzapine in schizophrenia". Clinical and Administrative Division, University of Iowa College of Pharmacy, Iowa City, Iowa, USA.

46. Scot E Purdon., *et al*. "Neuropsychological Change in Early Phase Schizophrenia During 12 Months of Treatment With Olanzapine, Risperidone, or Haloperidol. The Canadian Collaborative Group for Research on Cognition in Schizophrenia". *Archives of General Psychiatry* 57.3 (2000): 249-258.

47. Daniel R., *et al*. "Implications of Normal Brain Development for the Pathogenesis of Schizophrenia". *Archives of General Psychiatry* 44.7 (1987): 660-669.

48. Antonio Di Ieva., *et al*. "The indusium griseum and the longitudinal striae of the corpus callosum". *Cortex* 62 (2015): 34-40.

49. M S Keshavan., *et al*. "Abnormalities of the corpus callosum in first episode, treatment naive schizophrenia". *Journal of Neurology, Neurosurgery & Psychiatry* 72.6 (2002): 757-760.

50. M Mallar Chakravarty., *et al*. "Striatal shape abnormalities as novel neurodevelopmental endophenotypes in schizophrenia: A longitudinal study". *Human Brain Mapping* 36.4 (2015): 1458–1469.

51. Thiago Pereira Rodrigues., *et al*. "Orbitofrontal sulcal and gyrus pattern in human: an anatomical study". *Arquivos de Neuro-Psiquiatria* 73.5 (2015): 431-435.

52. Conrad Andrew ., *et al*. "Schizophrenia and the Hippocampus: The Embryological Hypothesis Extended". *Schizophrenia Bulletin* 13.4 (1987): 577-587.

53. Susan M Bowyer., *et al*. "Potential Biomarkers of Schizophrenia from MEG Resting-State Functional Connectivity Networks: Preliminary Data". *Journal of Behavioral and Brain Science* 5.1 (2015).

54. Kelvin O Lim. "Connections in Schizophrenia". *The American Journal of Psychiatry* 164.7 (2007): 995-998.

55. Science Daily (Feb. 24, 2010): Mouse Model May Provide Insight Into the Schizophrenicbrain.

56. Sang-Hun Lee., *et al*. "Efficient generation of midbrain and hindbrain neurons from mouse embryonic stem cells". *Nature Biotechnology* 18 (2000): 675 - 679.

57. Chen DQ., *et al*. "Age-Related Changes in Diffusion Tensor Imaging Metrics of Fornix Subregions in Healthy Humans". *Stereotactic and Functional Neurosurgery* 93.3 (2015): 151-159.

58. Haiyun Xu., *et al*. "Oligodendrocytes in Schizophrenia". Department of Anatomy, Southern Illinois University Carbondale, Carbondale, IL 62901, USA (2011).

59. Samsom JN., *et al*. "Schizophrenia and Depression Co-Morbidity: What We have Learned from Animal Models". *Front Psychiatry* 18 (2015).

60. Ridha Joober., *et al*. "Genetics of schizophrenia: from animal models to clinical studies". *Journal of Psychiatry & Neuroscience* 27.5 (2002): 336–347.

61. Johns Hopkins Medical Institutions : First Mouse Model Of Schizophrenia Developed. Science Daily. Retrieved March 15, 2012.

62. Hreinn Stefansson., *et al*. "Common variants conferring risk of schizophrenia". *Nature* 460.7256 (2009) 744-747.

63. Sigurdsson T., *et al*. "Impaired hippocampal-prefrontal synchrony in a genetic mouse model of schizophrenia". *Nature* 464.7289 (2010): 763-767.

64. 64-Kenneth S Kendler., *et al*. "The Genetics of Schizophrenia: Chromosomal Deletions, Attentional Disturbances, and Spectrum Boundaries". *The American Journal of Psychiatry* 160.9 (2003): 1549-1553.

65. Y Kohn1., *et al*. "Excitement and confusion on chromosome 6q: the challenges of neuropsychiatric genetics in microcosm". *Molecular Psychiatry* 10.12 (2005): 1062–1073.

66. Amanda Leigh Mascarelli: Faulty connectivity found in mouse model of schizophrenia(18 June 2010)Denver, Colorado.

67. Linda M Brzustowicz., *et al*. "Location of a Major Susceptibility Locus for Familial Schizophrenia on Chromosome 1q21-q22". *Science* 288.5466 (2000): 678-682.

68. ROBIN SHERRINGTON., *et al*. "Localization of a susceptibility locus for schizophrenia on chromosome 5". *Nature* 336.6195 (1988): 164-167.

69. Richard E Straub., *et al*. "A potential vulnerability locus for schizophrenia on chromosome 6p24–22: evidence for genetic heterogeneity". *Nature Genetics* 11.3 (1995): 287-393.

70. Robert Freedman., *et al*. "Linkage of a neurophysiological deficit in schizophrenia to a chromosome 15 locus". *Proceedings of the National Academy of Sciences* 94.2 (1997): 587-592.

71. Jean-Louis Blouin., *et al*. "Schizophrenia susceptibility loci on chromosomes 13q32 and 8p21". *Nature Genetics* 20.1 (1998): 70-73.

72. HOVATTA I., *et al*. "Linkage analysis of putative schizophrenia gene candidate regions on chromosomes 3p, 5q, 6p, 8p, 20p and 22q in a population-based sampled Finnish family set". *Molecular Psychiatry* 3.5 (1998): 452-457.

73. M J Owen., *et al*. "The molecular genetics of schizophrenia: new findings promise new insights". *Molecular Psychiatry* 9.1 (2004): 14-27.

74. P. Alexander Arguello., *et al*. "Cognition in Mouse Models of Schizophrenia Susceptibility Genes". *Schizophrenia Bulletin* 36.2 (2010): 289-300.

75. Papaleo F., *et al*. "Mouse models of genetic effects on cognition: relevance to schizophrenia". *Neuropharmacology* 62.3 (2012): 1204-1220.

76. German Torres ., *et al*. "Animal Models for Schizophrenia: The chakragati Mouse Mutant". *Kopf Carrier* (2008).

77. Stephen J., *et al*. "Association between a functional catechol O-methyltransferase gene polymorphism and schizophrenia". *The American Journal of Psychiatry* 160.3 (2003): 469-476.

78. Torfi Sigurdsson., *et al*. "Impaired hippocampal–prefrontal synchrony in a genetic mouse model of schizophrenia". *Nature* 464.7289 (2010): 763-767.

79. Modeling Schizophrenia Phenotypes—DISC1 Transgenic Mouse Debuts (2007).

80. Michael J., *et al*. "Dysbindin-1 and schizophrenia: from genetics to neuropathology". *Journal of Clinical Investigation* 113.9 (2004): 1255-12557.

81. Penn Team Links Schizophrenia Genetics to Disruption in How Brain Processes Sound.

82. Tsuyoshi Miyakawa., *et al*. "Conditional calcineurin knockout mice exhibit multiple abnormal behaviors related to schizophrenia". *Proceedings of the National Academy of Sciences of united states of America* 100.15 (2003): 8987-8992.

83. Taisuke Kato., *et al*. "Phenotypic Characterization of Transgenic Mice Over expressing Neuregulin". *PloS one* 5.12 (2010).

84. Amanda Law., *et al*. "Schizophrenia, IV: Neuregulin-1 in the Human Brain". *American Journal of Psychiatry* 160.8 (2003): 1392.

85. Dawei Li., *et al*. "Meta-analysis shows strong positive association of the neuregulin 1 (NRG1) gene with schizophrenia 1Bio-X Center and NHGG". *Human molecular genetics* 15.12 (2006): 1995-2002.

86. Investigators Create Schizophrenia ,GEN News Highlights: Dec 29, 2009.

87. Medical College of Georgia scientists, Science Daily (Dec. 28, 2009): reducing the inhibition of brain cells involved in complex reasoning and decisions about appropriate social behavior , published Dec. 28 in the Proceedings of the National Academy of Sciences.

88. Zhenqi Wang., *et al*. "A Review and Re-Evaluation of an Association Between the NOTCH4 Locus and Schizophrenia". *American Journal of Medical Genetics Part B* 141.8 (2006): 902-906.

89. Jun Wei., *et al*. "A review and re-evaluation of an association between the NOTCH4 locus and schizophrenia". *American Journal of Medical Genetics Part B (Neuropsychiatric Genetics)* 141B.8 (2006): 902-906.

90. Vuillermot., *et al*. "Schizophrenia-relevant behaviors in a genetic mouse model of constitutive Nurr1 deficiency(2011)". *Gene brain and behaviour* 10.5 (2011): 589-603.

91. Yun-Hsiang Chen., *et al*. "Mutation analysis of the human NR4A2 gene, an essential gene for midbrain dopaminergic neurogenesis, in schizophrenic patients". *American Journal of Medical Genetics* 105.8 (2001): 753-757.

92. ILIA Maria., *et al*. "Expression of Oct-6, a POU III domain transcription factor, in schizophrenia".

93. *The American Journal of Psychiatry* 159.7 (2002): 1174-1182.

94. Kirenjee Ubhietal., *et al*. "Expression of POU-domain transcription factor, Oct-6, in schizophrenia, bipolar disorder and major depression". *BMC Psychiatry* 24.5 (2005): 38.

95. DeSilvaU., *et al*. "The human reelin gene: isolation, sequencing, and mapping on chromosome 7". *Genome Research* 7.2 (1997): 157-164.

96. Tremolizzo L., *et al*. "An epigenetic mouse model for molecular and behavioral neuropathologies related to schizophrenia vulnerability". *Proceedings of the National Academy of Sciences* 99.26 (2006): 17095-17100.

97. Gavin S Dawe., *et al*. "Pathophysiology and Animal Models of Schizophrenia". *ANNALS Academy of Medicine Singapore* 38.5 (2009): 425-426.

98. Mirnics K., *et al*. "Disease-specific changes in regulator of G-protein signaling 4 (RGS4) expression in schizophrenia". *Molecular Psychiatry* 6.3 (2001): 293-301.

99. Oliver PL., *et al*. "Disrupted circadian rhythms in a mouse model of schizophrenia". *Current Biology* 22.4 (2012): 314-319.

100. Asada M., *et al*. "Reduced tumor growth in a mouse model of schizophrenia, lacking the dopamine transporter". *International Journal of Cancer* 123.3 (2008): 511-518.

101. Jason Tilan and Joanna Kitlinska. "Sympathetic Neurotransmitters and Tumor Angiogenesis—Link between Stress and Cancer Progression". *Journal of Oncology* (2010).

102. Alan R. Sanders., *et al*. "No Significant Association of 14 Candidate Genes With Schizophrenia in a Large European Ancestry Sample: Implications for Psychiatric Genetics". *American Journal of Psychiatry* .165.4 (2008): 497-506.

103. Taisuke Kato., *et al*. "Phenotypic Characterization of Transgenic Mice Over expressing Neuregulin". *PloS one* 5.12 (2010).

104. Bridget Valsamis, Susanne Schmid: Habituation and Prepulse Inhibition of Acoustic Startle in Rodents, Anatomy and Cell Biology, Schulich School of Medicine and Dentistry, University of Western Ontario .

105. Martha E. Shenton., *et al*. "A review of MRI findings in schizophrenia". *Schizophrenia Research* 49.1-2 (2001): 1-52.

106. Ulrich Ettinger., *et al*. "Smooth pursuit and antisaccade eye movements in siblings discordant for schizophrenia". *Journal of Psychiatric Research* 38.2 (2014): 177-184.

107. BurkeJ G ., *et al*. "Improved antisaccade performance with risperidone in schizophrenia Neuropsychopharmacology Unit". *Journal of Neurology, Neurosergery and Psychiatry* 72.4 (2002): 449-454.

108. LiddleP F., *et al*. "Patterns of cerebral blood flow in schizophrenia .Department of Psychological Medicine". *British Journal of psychiatry* 160 (1992): 179-186.

109. Daniel R., *et al*. "Physiologic Dysfunction of Dorsolateral Prefrontal Cortex in Schizophrenia, Regional Cerebral Blood Flow Evidence". *Arch Gen Psychiatry* 43.2 (1986): 114-124.

110. CB Mikell., *et al*. The Hippocampus and Nucleus Accumbens as Potential Therapeutic Targets for Neurosurgical Intervention in Schizophrenia". *Stereotactic and Functional Neurosurgery* 87.4 (2009): 256-265.

111. BD Greenberg., *et al*. "Mechanisms and the Current State of Deep Brain Stimulation in Neuropsychiatry". *CNS Spectrums* 8.7 (2003): 522-526.

112. J Kuhn., *et al*. "Deep Brain Stimulation for Psychiatric Disorders Psychiatry and psychotherapy clinic". *Dtsch Arzteblatt International* 107.7 (2010): 105-113.

113. Kopell Brian Harris., *et al*. "Deep Brain Stimulation for Psychiatric Disorders". *Journal of Clinical Neurophysiology* 21.1 (2004): 51-67.

114. Roy A. "Deep Brain Stimulation for Schizophrenia". *Functional Neurosurgery* 87.4 (2009): 266.

115. Alan Anderson and Arciniegas DB. "Neurosurgical interventions for neuropsychiatric syndromes". *current psychiatric reports* 6.5 (2004): 355-363.

116. DK Binder and Iskandar BJ. "Modern Neurosurgery for Psychiatric Disorders". *Neurosurgery* 47.1 (2000): 21-23.

117. Anthony Wright (1997)Chapter 5: Limbic System: Hippocampus, Department of Neurobiology and Anatomy, The UT Medical School at Houston.

118. George W F., *et al.* "Neurosurgery for mental disorder: past and present Advances in Psychiatric Treatment". *Neurology* . 5 (1999): 261-270.

119. Wang Yifang., *et al*. "Treatment of intractable schizophrenia with stereotactic surgery". *chinese Journal of Stereotactic an functional Neurosurgery* 2006-2005.

120. Ballantine HT Jr., *et al*. "Treatment of psychiatric illness by stereotactic cingulotomy". *Biol Psychiatry* 22.7 (1987): 807-819.

121. JW Leiphart., *et al*. "Stereotactic lesions for the treatment of psychiatric disorders". *Journal of Neurological Surgery* 113.6 (2010): 1204-1211.

122. Wang Xiaofeng., *et al*. "Treatment of intractable schizophrenia of I type with stereotactic techniques". *chinese Journal of Stereotactic an functional Neurosurgery* 2004-2006.

123. Geoffery K., *et al*. "Stereotactic tractotomy in the surgical treatment of mental illness , From the Department of Surgery". *Journal of Neurology, Neurosergery and Psychiatry* 28.4 (1965): 304-310.

124. AL Malizia., *et al*. "psychiatric disorders Psychopharmacology Unit, University of Bristol BS8 1TD and MRC Cyclotron Unit". *Journal of Psychopharmacology* 11.2 (1997): 179-187.

125. Titus Lanz., *et al*. "A High-Throughput Eight-Channel Probe Head for Murine MRI at 9.4 T". *Magnetic Resonance in Medicine* 64.1 (2010): 80-87.

126. Ian Rowland., *et al*. "Stereotactic Device for Animal MRI of the Radiology Department at UW-Hospital BME 201 University of Wisconsin" (2008).

127. Sumeer L., *et al*. "An implantable guide-screw system for brain tumor studies in small animals". *Journal of Neurosurgery* 92.2 (2000): 326-333.

128. Shinya Yamada., *et al*. "A method to accurately inject tumor cells into the caudate/putamen nuclei of the mouse brain". *Tokai journal of experimental and clinical medicine* 29.4 (2004): 167-173.

129. Ramrath L., *et al*. "Intelligent Robots and Systems". 2007. IROS 2007. IEEE/RSJ International Conference , (2007): 859-864.

130.   Clémentine Bosch., *et al*. "Subthalamic nucleus high-frequency stimulation generates a concomitant synaptic excitation–inhibition in substantia nigra pars reticulata1Dynamics and Pathophysiology of Neuronal Networks (INSERM U-1050)". *Journal of psycology* 589. Pt 17 (2011): 4189-4207.

131.   T Tokay., *et al*. "High-frequency magnetic stimulation induces long-term potentiation in rat hippocampal slices". *Neuroscience letters* 461.2 (2009): 150-154.

132.   Kongkham PN., *et al*. "Complications in 622 cases of frame-based stereotactic biopsy, a decreasing procedure". *Canadan journal of Neurological sceince* 35.1 (2008): 79-84.

133.   Christopher A Ross., *et al*. "Neurobiology of Schizophrenia" *Neuron* 52.1 (2006): 139-153.