Schizophrenia; a neurotransmitter model

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Abstract

Schizophrenia is a mental disorder which we are currently beginning to understand. Performed research has allowed a neurotransmitter model to be formulated which could explain the positive symptoms of schizophrenia. In this thesis a literature study has been performed to investigate the role of neurotransmitters in the origin of positive symptoms in schizophrenia. Hypofrontality is the cause of both a hypoactive dopaminergic mesocortical projection and a hyperactive dopaminergic mesolimbic projection. The hypoactive mesocortical projection seems to be the cause of the negative symptoms of schizophrenia, while the hyperactive mesolimbic projection appears to be the cause of the positive symptoms of schizophrenia. The hyperactive mesolimbic projection reduces latent inhibition. This could cause a sensory overload of the cortex which could produce positive symptoms. These dopamine projections seem to be regulated by a system involving both glutamate and GABA. However, this model does not explain everything about schizophrenia. Therefore more research should be performed to fully understand schizophrenia.

Keywords: Schizophrenia, Positive symptoms, mesolimbic projection, hypofrontality, dopamine, glutamate, GABA

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Introduction

Mental disorders are a global problem. Annually, billions are spent on medical aid for those with mental problems. Schizophrenia is a mental disorder which affects approximately 1% of the global population. It has been estimated that in the United States the costs of schizophrenia exceeds those of all cancers together [1]. In 2002, the total costs of schizophrenia in the United States have been estimated at 67,2 billion. This estimate includes health care but also non-health care costs (i.e. law enforcement, homeless shelters, and research/training related to schizophrenia) [2]. Schizophrenia has already been described in ancient writings and thus has been around for centuries [3]. In addition, the symptoms of schizophrenia are equal in a wide variety of cultures all around the world [4]. This means that schizophrenia is not something of the last decades and that schizophrenia is a universal disease.

Schizophrenia literally means “split mind”, but it does not imply a split or multiple personality. Eugen Bleuler, who introduced the term schizophrenia, meant to refer to disorganization of the mind which causes a “break” with reality, such that thoughts and feelings do not interact in a normal way.

Schizophrenia is a lifelong disease for most patients. Once you get the disease, the chances are likely you never fully recover [5]. After 10 years, 25% of the people diagnosed with schizophrenia are fully recovered, 25% have improved a lot and are relatively independent, 25% of the schizophrenia patients have improved but require an extensive support network, 15% is hospitalized and unimproved and 10% is deceased, of which most have committed suicide [5].

Symptoms

Symptoms of schizophrenia can be subdivided into three categories: positive, negative and cognitive [6]. Positive symptoms reflect the presence of abnormal thoughts and behaviours. Positive symptoms can include: thought disorders, hallucinations and delusions [6]. The experience of thought disorders, hallucinations and delusions could also be referred to as psychosis, but usually a psychosis is a single experience of these positive symptoms while in schizophrenia the patients experience the positive symptoms more often and in an episodic manner [6].

A thought disorder (disorganized, irrational thinking) is one of the most important symptoms of schizophrenia [6, 7]. Schizophrenia patients have a lot of trouble with arranging their thoughts logically and deciding which thoughts are plausible and which are absurd [7]. In a conversation, a schizophrenia patient can jump from one topic to the next based on some vague association. Sometimes schizophrenia patients choose words for rhyme instead of meaning or use meaningless words.

The second positive symptom of schizophrenia is hallucinations, the perception of stimuli which are not present [6]. The most common hallucinations are auditory hallucinations, but the hallucinations can be in any of the senses [6]. Often, the hallucinations encompass voices which give orders to the schizophrenia patient, scold the schizophrenia patient for his/her unworthiness or talk gibberish.

The third positive symptom of schizophrenia is the delusion. Delusions are false beliefs and contradict reality [6]. Several types of delusions can be distinguished. The most common delusions are delusions of persecution, control and grandeur [6]. Delusions of persecution are false beliefs of someone following oneself, or conspiring and plotting against you. Delusions of control, which are related to the persecution delusion, encompass false

beliefs in that the patient thinks he/she is being controlled by others, for example by means of a subcutaneous chip. Delusions of grandeur are false beliefs in one's power and importance, for example beliefs that the patient is godlike or has special and unique knowledge that no one else has.

Negative symptoms reflect the absence of normal responses or behaviours which are normally present. These symptoms encompass: flattened emotional responses, poverty of speech, lack of initiative and persistence, anhedonia (inability to experience pleasure) and social withdrawal [6]. Cognitive symptoms include difficulty in sustaining attention, reduced psychomotor speed (the ability to rapidly and fluently make movements of the extremities), memory impairment, poor abstract thinking and poor problem solving [6]. The negative and cognitive symptoms are not only present in schizophrenia, but are also seen in patients with brain damage, especially in the frontal lobes [8-11].

The symptoms gradually appear over approximately three to five years [6]. The first symptoms to appear are the negative symptoms, followed by cognitive symptoms and the positive symptoms are the last to appear, often several years later [6]. The onset differs per gender; schizophrenia usually begins to appear around the age of 18 in males and 25 in females [12]. There are indications that subtle signs of schizophrenia are present at earlier ages; however, these early signs are not very specific [6]. It is even thought that the process of pathogenesis perhaps already starts during prenatal period [6]. This process is influenced by both environmental and genetic factors [6].

The past few decades much progress has been made regarding the cause of schizophrenia. However, we are currently just beginning to understand schizophrenia and its causes. The neurobiological underpinnings of positive symptoms are still largely unknown. Of all symptoms, the positive symptoms are most unique for schizophrenia; negative and cognitive symptoms are also present in other pathologies, for example in depression or dementia [13].

Dopamine hypothesis

A role of dopamine in schizophrenia was discovered largely by accident. In 1950 the French surgeon Laborit thought that an antihistamine could be effective in calming down patients before surgery, because during surgeries dangerous amounts of histamine can be released into the circulation [14]. The first results of administration of the antihistamine promethazine were very satisfactory [14]. A derivative of promethazine, chlorpromazine appeared to have no antihistaminergic activity, but it turned out to be an antipsychotic when it was given to schizophrenia patients [15]. Chlorpromazine was the first successful medicine which could reduce the symptoms of schizophrenia. However, chlorpromazine only reduced positive symptoms and had many side effects, some of which looked a lot like Parkinson's disease (extrapyramidal side effects) [16]. When the Austrian neurologist Hornykiewicz looked at the brains of deceased Parkinson patients he discovered that the dopamine levels were very low in these brains [17]. The correlation between Parkinson and dopamine soon led to speculations about the antipsychotic effect of chlorpromazine.

It was the Swede Arvid Carlsson who discovered that chlorpromazine raised homovanillic acid (HVA, a dopamine metabolite) concentrations [18]. He also found that HVA concentrations correlated with the clinical potency of antipsychotics. In 1963, the hypothesis that antipsychotics block dopamine receptors was postulated. This hypothesis was described as a negative feedback mechanism (increased dopamine release leads to reduced dopamine

transmission) [18]. This hypothesis has been confirmed in 1975 using the receptor-binding assay [19]. The (presynaptic) dopamine D_2 receptor seemed responsible for the negative feedback effect [20]. Antipsychotics block the autoreceptors and this leads to enhanced dopamine release. However, antipsychotics also block postsynaptic receptors, and because of this the effect dopamine transmission also diminishes as result of antipsychotics. After chronic treatment the firing rate of dopamine cells diminishes while the antipsychotics still block the postsynaptic receptors [21], thus antipsychotics reduce dopamine transmission.

The discovery of the working mechanism of antipsychotics led to the formulation of the dopamine hypothesis (mainly based on the effects of antipsychotics) [22]. According to this dopamine hypothesis, positive symptoms in schizophrenia are caused by hyperactivity of the dopamine system. This hypothesis was supported by the fact that amphetamines, which strongly enhance dopamine transmission, could induce a psychosis in schizophrenia patients [23].

However, it soon became clear that this dopamine hypothesis was not formulated properly. Antipsychotics were not effective in alleviating positive symptoms in all schizophrenia patients and if they worked they only did so after chronic treatment [24]. In addition, non-schizophrenia patients usually do not, if ever, develop psychotic symptoms from a single usage of drugs which enhance dopamine transmission [23]. Moreover, from measurements in post-mortem brains [25-30] and cerebrospinal fluid (CSF) [31] it appears that there was no support for homogenous hyperactivity of the dopamine system. One finding was found consistently in these studies: HVA concentrations correlated negatively with cortical atrophy and ventrical enlargement [25-31], which are both present in schizophrenia patients [6]. Since schizophrenia patients have reduced HVA concentrations, there could be hypoactivity instead of hyperactivity of the dopamine system in schizophrenia patients.

Research question

The dopamine hypothesis has dominated schizophrenia research for some decades. However, lately it has been shown that glutamate and γ-aminobutyric-acid (GABA) might be related to the origin of the positive symptoms as well [32]. It appears that the negative symptoms could be related to the cause of the positive symptoms [21], therefore the negative symptoms will be discussed as well. Some first steps have been made to try to integrate neurotransmitters in a single “schizophrenia neurotransmitter model”. I will try to answer the question if there is a model involving dopamine, GABA and glutamate and if this model could explain the positive symptoms of schizophrenia.

Dopamine

Dopamine hyperactivity

Much research has been dedicated to resolving the contradictory results regarding dopamine transmission in schizophrenia. Differences in dopamine and HVA concentrations in patients compared to controls have been found in post-mortem studies. For example, in patients the concentrations of HVA have been found to be higher in the striatum, more specifically the nucleus accumbens (part of the (ventral) striatum) [33]. This global increase of HVA concentration in the striatum was attributed to the antipsychotic treatment. Another study found an increase in dopamine concentrations in the nucleus accumbens [34]. In addition, a study that looked at receptor occupancy found an increase in dopamine in the striatum which was independent of antipsychotic treatment [35]. This increase in dopamine levels was found during illness exacerbation.

Post-mortem studies showed differences in D_2 receptor prevalence in the striatum in patients [33, 34, 36]. This increase in D_2 receptor prevalence was not simply the consequence of antipsychotic treatment [36]. In animal studies, antipsychotic treatment increased the amount of D_2 receptor with 30%, while schizophrenia patients have 50-60% more D_2 receptors than controls [33, 34, 36-38]. Alzheimer and Huntington patients treated with antipsychotics had an increase of 25% in their striatal amount of D_2 receptors compared to an increase of over 100% in schizophrenia patients [36]. This all strongly suggests that the antipsychotic treatment only partially explains the increase in D_2 receptor numbers.

Dopamine is not randomly distributed in the brain. The dopamine system can be subdivided in three main projections based on anatomy: nigrostriatal, mesolimbic and mesocortical (*figure 1*). The nigrostriatal system is a projection of the substantia nigra (also called A9) to the striatum (i.e. putamen, caudate nucleus). This projection is involved in the regulation of movements. The cell bodies of the mesolimbic projection are in the ventral tegmental area (also called A10) of the midbrain and partially in the substantia nigra. These nuclei project to the nucleus accumbens, olfactory tubercle and amygdala. The mesocortical projection has its cell bodies mainly in A10. This system projects mainly to the prefrontal cortex, but also to the nucleus accumbens, septum and olfactory tubercles [39, 40].

It appears that different antipsychotics have different effects on these systems. A single dose of a “typical antipsychotic” (i.e. haloperidol) increases dopamine neuron firing in the nigrostriatal and mesolimbic dopamine systems [41, 42]. Chronic administration of antipsychotics reduces dopamine neuron activity

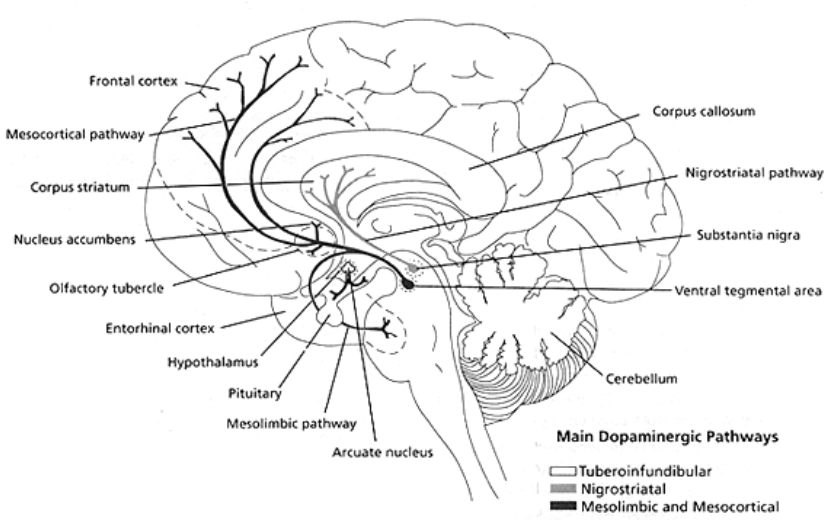

Figure 1. The different dopamine projections are shown: tuberoimfundibular, nigrostriatal, mesolimbic and mesocortical. The projections are shown based on their nucleus of origin.

to below pretreatment levels [43, 44]. The D_2 receptor blocking effect of the antipsychotics is responsible for disturbances in negative feedback [42, 43]. Atypical antipsychotics, antipsychotics which do not cause the extrapyramidal side effects, (for example Clozapine) affect only A10 and are thus more selective in its effects [40-49]. These data led to the hypothesis that A9 is responsible for the extrapyramidal side effects while A10 seems to be responsible for the antipsychotic effects of the antipsychotics [46]. These data suggests that a more localized increase in dopamine activity is responsible for a psychosis and more specifically an increase of activity of the dopamine neurons in the ventral tegmental area.

This hypothesis has been validated in humans using plasma HVA measurements. A positive correlation was found between clinical severity and plasma HVA levels [50-52]. However, the schizophrenia patients still had a lower average of plasma HVA levels compared to controls. There is a paradox here, namely dopamine levels correlate positively with clinical severity in schizophrenia but patients have lower dopamine levels compared to controls.

Not only differences in dopamine levels in different brain regions are a characteristic of schizophrenia, there also appears to be a difference in amphetamine-induced dopamine release in schizophrenia patients compared to controls. It has been found that amphetamine induces a greater dopamine release in schizophrenia patients [35, 53]. This increase in dopamine release is not caused by a difference in amphetamine distribution [35] or dopamine terminal density [54]. Moreover, the use of antipsychotics does not influence this amphetamine-induced dopamine release difference [35]. However, a difference seems to exist amongst schizophrenia patients. Schizophrenia patients who experience symptom exacerbation showed a greater amphetamine-induced dopamine release than schizophrenia patients who did not show symptom exacerbation and the latter did not even show an increased dopamine release compared to controls [35]. These data suggest that dysregulation of the dopamine system might be present only during periods of exacerbation.

These data indicate that there is dopamine hyperactivity in schizophrenia patients. This increase in dopamine transmission however, seems to be present only during a period of exacerbation. The mesolimbic system seems to be the cause of this increase in dopamine transmission, with the projections to the nucleus accumbens as the major site of increased dopamine transmission. Nevertheless, these data alone do not resolve the paradox present in schizophrenia patients: lower levels of dopamine in the plasma of schizophrenia patients compared to controls while the levels of dopamine are positively correlated with clinic severity. Moreover, how does this dopamine hyperactivity in the nucleus accumbens lead to positive symptoms?

Nucleus Accumbens

Hyperactivity of the mesolimbic projection leads to positive symptoms. How does dopamine hyperactivity in the nucleus accumbens lead to positive symptoms? It has been shown that the nucleus accumbens is involved in latent inhibition [55]. Latent inhibition is “a loss of associability that occurs when a stimulus is simply presented a number of times without further consequence” [55]. Dopamine release in the nucleus accumbens has been shown to disrupt latent inhibition [55]. Stimuli that are normally ignored are thus noticed if there is an increase in dopamine release in the nucleus accumbens. This sounds a little like a hallucination, since in a hallucination stimuli are noticed that are in fact not present. Thus, stimuli are noted that should not be noted. The hippocampus also sends (probably

pyramidal glutamate) projections to the nucleus accumbens that are involved in latent inhibition [55] When these projections are abolished latent inhibition is also disrupted [55]. This means that this hippocampal projection normally stimulates latent inhibition.

The thalamus is a relay station for all senses [56]. Considering latent inhibition, it is likely that the nucleus accumbens is involved in the filtering function of the thalamus [56]. It is thought that GABAergic neurons, which belong to the striatothalamic projection, exert an inhibitory influence on thalamocortical glutamatergic neurons [56]. This inhibitory influence aids in filtering useless information in the thalamus, therefore protecting the cortex from a sensory overload [56]. It is thought that the excessive dopamine in the nucleus accumbens inhibits the activity of the GABAergic striatothalamic neurons (D_2 receptor is inhibitory) which thus leads to a higher activity of the thalamocortical neurons [56]. This increased amount of sensory information could induce a sensory overload which could expresses itself as a psychosis [56]. Thus, increased dopamine activity of in the nucleus accumbens could lead to a sensory overload in the cortex.

Dopamine hypoactivity

Schizophrenia is characterized by poor social functioning [6]. Observations in primates suggest that diminished cortical functioning is responsible for poor social functioning. Monkeys that sustain a frontal lobe ablation have problems with suppressing irrelevant stimuli, concentration and social functioning. This decreased social functioning is reminiscent of schizophrenia. These monkeys get chased from their group by other animals and die in isolation [57]. Also, these symptoms, which look a lot like the negative symptoms of schizophrenia, appear only after those monkeys have reached the monkey equivalent of adolescence [58, 59] just as it happens in humans.

Frontal lobe dysfunction, or in this case: hypofrontality (decreased activity of the prefrontal cortex), has been shown to be present in schizophrenia patients in the form of decreased cerebral blood flow [60-62]. Mesocortical dopamine neurons have less inhibitory autoreceptors compared to other dopamine neurons and therefore the dopamine turnover in these neurons is greater than in mesolimbic neurons [63]. Since the cortex has such a large volume and a good venous drainage compared to the striatum in a human brain, the brain contribution to CSF and plasma levels of HVA reflects mainly cortical dopamine activity [21]. Cortical dopamine mainly represents frontal dopamine activity, because dopamine is mainly present in the frontal cortex (mesocortical projection) (where mainly D_1 receptors reside) compared to other cortical regions [64]. These data in combination with the earlier results of a negative correlation between HVA levels and cortical atrophy and ventricle enlargement [25-31] indicate that hypofrontality could be caused by diminished mesocortical dopamine activity.

That hypofrontality is caused by low cortical dopamine activity is suggested by the finding that a lack of increase in cerebral blood flow during a prefrontal task is strongly correlated with low CSF HVA concentrations [65]. Administration of amphetamine increases blood flow in the prefrontal cortex in schizophrenia patients, which suggests that the hypofrontality can be diminished by increasing prefrontal dopamine [66]. This increase in prefrontal blood flow after administration of amphetamine is also correlated with the improvement seen in a prefrontal task [67].

These data suggest that schizophrenia patients have decreased prefrontal dopamine activity. The negative symptoms seem to be the main consequence of this hypofrontality. Because there are mainly D_1 receptors and there are barely D_2 receptors present in the

prefrontal cortex, atypical antipsychotics (D_2 antagonists) do not improve these negative symptoms.

Recapitulation

Schizophrenia appears to be more difficult to understand now that we know more about it. The initial idea was that schizophrenia is caused by a "simple" hyperactivity of the dopamine system, but studies show that the picture is not that simple. Mesolimbic projections from the ventral tegmental area to the nucleus accumbens seem to be hyperactive and associated with positive symptoms, while the mesocortical projections seem to be hypoactive and associated with negative symptoms. The hyperactive mesolimbic projection could lead to a sensory overload in the cortex. The out of balance dopamine systems appear to be most prominent during exacerbation. The question remains what causes this unbalanced dopamine systems. Evidence points in the direction of glutamate and GABA [68].

Glutamate

Ketamine

More than 50 years ago, ketamine is introduced as a dissociative anesthetic. Ketamine is thought to induce these effects through a blockage at the same site of an ionotropic glutamate receptor; the NMDA (n-methyl-d-aspartic acid) receptor [69]. It was soon known to cause a psychotic syndrome that could hardly be distinguished from schizophrenia [70, 71]. In addition, just as in schizophrenia, children do not show this psychotic syndrome [72]. Besides producing psychotic symptoms, ketamine also produces physiological signs of schizophrenia in non-schizophrenic volunteers. For example, schizophrenia patients and their first-degree relatives show eye-tracking abnormality [73] and after receiving ketamine healthy people also display abnormal saccades [74]. It was also shown that steady low doses of ketamine induced negative symptoms, cognitive impairments and some positive symptoms in non-schizophrenic volunteers [75]. Moreover, it has been shown that declarative memory (memory of facts and events), which is also impaired in schizophrenia, was affected by ketamine [76]. Ketamine can also induce thought disorders [77]. Non-schizophrenic volunteers show an enhanced amphetamine induced dopamine release when they are given ketamine, similar to schizophrenia patients (without the ketamine) [78]. When schizophrenia patients were given ketamine they were shown to be quite sensitive to the psychotomimetic effects of ketamine [79].

These data show that ketamine induces symptoms that look very similar to schizophrenia. This means that NMDA hypofunction could lead to symptoms of schizophrenia. This NMDA hypofunction also affects dopamine transmission; it is even thought that the dopamine dysregulation is secondary to the NMDA hypofunction [78].

Glycine modulatory site

If the NMDA receptor is hypoactive in schizophrenia, then where does this hypoactivity come from? Kynurenic acid is an endogenous glycine modulatory site (GMS) antagonist. The GMS is a binding site on the NMDA receptor which needs to be occupied in order for the NMDA receptor to open the ion-channel [80]. It has been shown that kynurenic acid levels are elevated in the prefrontal cortex but not in the motor cortex in schizophrenia patients [81]. Kynurenic acid levels have also shown to be elevated in the CSF of schizophrenia patients [82, 83]. Moreover, the enzyme tryptophan-2, 3-dioxygenase, an

upstream enzyme in the kynurenic acid synthesis pathway, is upregulated in the frontal cortex of schizophrenia patients [84]. This increase in endogenous kynurenic acid has been shown to induce a sensory gating abnormality in rats, which is also common in schizophrenia. [85].

Kynurenic acid is not the only endogenous GMS antagonist that seems to be increased in schizophrenia. Glutamate carboxypeptidase II (GCP II) was shown to have a lower expression in the prefrontal cortex [86, 87], hippocampus and temporal cortex [88] in schizophrenia patients compared to controls. GCP II catabolyzes n-acetyl-aspartyl glutamate (NAAG) to glutamate [88]. NAAG is a metabotropic glutamate receptor 3 (mGluR3) agonist [89, 90] as well as a glycine reversible NMDA receptor antagonist [91, 92]. Stimulating the mGluR3 down regulates glutamate release (negative feedback) [93]. This means that the reduced expression of GCP II would have reduced glutamate levels in the prefrontal cortex as a consequence, but also increase NMDA receptor blockage due to increased NAAG levels. These data indicate that the NMDA hypoactivity is partially explained by increased endogenous NMDA receptor antagonists and perhaps even reduced glutamate. These data also suggest that this NMDA receptor problem resides in the prefrontal cortex.

If the NMDA receptor is hypoactive in schizophrenia patients, then stimulating the NMDA receptor might relieve symptoms in schizophrenia patients. Direct activation of the NMDA receptor could lead to excitotoxicity and neuronal degeneration [80]. An NMDA receptor activation leads to potassium and calcium influx, and too much calcium is toxic [80]. An indirect approach to increase NMDA receptor activity is to stimulate the GMS. This does not lead to tonic NMDA receptor activation, but this leads to an increased NMDA receptor response to endogenous released glutamate [80]. It has been shown that the GMS was not saturated by endogenous agonists [94-96] and could thus be used as a target to increase NMDA receptor activity.

Positive results have been found regarding GMS stimulation. Improvement of negative symptoms and cognitive symptoms has been found when glycine was given to schizophrenia patients while they were also given typical antipsychotics [97-99]. If d-serine (the most potent known GMS agonist [80]) was given to schizophrenia patients who were also receiving typical antipsychotics the schizophrenia patients showed improvement in cognition and reduced negative and positive symptoms [100, 101]. If schizophrenia patients received sarcosine next to typical antipsychotics they also showed an improvement of cognition and a reduction of negative and positive symptoms [102]. Sarcosine is an inhibitor of the glycine transporter 1 (GlyT1) which regulates synaptic glycine by actively pumping glycine from the synaptic cleft into the astrocyte on which GlyT1 resides [103]. These data suggest that GMS stimulation, and thus enhanced NMDA receptor activity, reduces negative and cognitive symptoms. If stimulation of the NMDA receptor is strong enough it appears that it could also reduce the positive symptoms in schizophrenia patients.

Clozapine

Clozapine is a unique antipsychotic. In a subpopulation of schizophrenia patients who respond poorly to typical antipsychotics clozapine reduces positive and negative symptoms [104] and also substance abuse [105-107]. These remarkable effects of clozapine appear to be due to indirect or direct stimulation of the GMS [108-110] combined with its effect on dopamine transmission [21]. This combination of effects makes clozapine a useful drug in treating schizophrenia.

Glutamate Hypofrontality

The data presented so far (mainly at the beginning of this chapter) regarding NMDA hypoactivity suggest that the NMDA hypoactivity is greatest in or localized mainly in the prefrontal cortex [81, 84, 86-88]. It has also been proposed that in the prefrontal cortex, temporal lobe and thalamus the glutamatergic projections are compromised [80, 111]. The axonal projections studied by using diffusion tensor imaging show a disruption of terminal fields in the prefrontal cortex [112]. This could indicate a "disconnection" in schizophrenia patients, and the notion about this disconnection is that primarily the glutamatergic afferents are affected [113]. These data indicate that mainly the glutamate neurons which reside in the prefrontal cortex are affected in schizophrenia patients. This decreased prefrontal glutamate activity could be what causes hypofrontality.

Genetics

Schizophrenia is a disease which has a high degree of genetic inheritance [6, 114]. Schizophrenia does not display a standard mendelian inheritance, but it is a disorder in which many genes have a small effect in determining the phenotype [115]. There are some genes known that contribute to a risk of developing schizophrenia, and most of these genes are involved in glutamate neurotransmission [116]. It should be noted that genes involved in dopamine and GABA transmission associated with a risk for schizophrenia have also been identified; however, more genes involved in glutamate neurotransmission have been found and have been replicated more often [116]. Examples of genes involved in glutamate neurotransmission that have been associated with a risk of developing schizophrenia are: G72 (gene product is involved in d-serine catabolization) [117], GRM3 (gene product is mGluR3) [118], DTNP1 [119] (gene product is involved in the vesicular release of glutamate [120]) and NRG1 [121] (gene product is involved in NMDA functioning [122]). These data suggest that glutamate is the neurotransmitter which is most often affected in schizophrenia by genetic variations. This could indicate that a glutamate transmission dysfunction could be the initial cause of schizophrenia and that the other neurotransmitters are deregulated due to glutamate dysfunction.

Recapitulation

Dopamine is no longer the only neurotransmitter that is being associated with schizophrenia, since glutamate has been shown be involved in schizophrenia as well. Studies with ketamine and PCP have shown the first evidence in the direction of glutamate involvement in schizophrenia and the NMDA receptor seems to be the major glutamate receptor involved. Stimulation of the GMS produces strong evidence of the NMDA receptor involvement and the GMS could be the perfect site for future NMDA receptor involved schizophrenia therapy. It seems that the GMS could already be used as a target site, as clozapine appears to use this GMS. Mild stimulation of the NMDA receptor reduces the negative and cognitive symptoms. Strong stimulation could reduce the positive symptoms. Moreover, the NMDA receptor hypofunction seems to be mainly present in the prefrontal cortex. The fact that hypofrontality seems to produce the negative symptoms indicates that the prefrontal glutamate neurons could be directly involved in this hypofrontality and could possibly even be the cause. The fact that only strong stimulation of the NMDA receptor has been associated with an improvement of positive symptoms indicates that the glutamate neurons have a more indirect effect regarding the positive symptoms. A possible mechanism could be that prefrontal glutamate projections stimulate the mesocortical dopamine projection and are (indirectly) involved in the activity of the mesolimbic

projection. Moreover, the fact that glutamate is the genetically most affected neurotransmitter in schizophrenia indicates that the glutamate (NMDA) hypoactivity could be a direct cause of schizophrenia.

GABA

GABA, the major inhibitory neurotransmitter in the mammalian brain is, like glutamate, involved in almost every system in the brain [123]. In the mammalian brain up to 30% of the cortical neurons use GABA [124]. In 1972, it was postulated that GABA was involved in schizophrenia [125]. It was thought that a defect of GABAergic neurons failed to regulate neural circuits involved in behavioural responses. This defect would exacerbate under stressful conditions in which a monoaminergic drive would increase activating input onto these GABA neurons which would produce abnormalities of perceptual and cognitive integration [125]. Ever since this hypothesis was formulated, it is thought that GABA is involved in schizophrenia [123].

GABAergic chandelier cell

There are two forms of glutamic acid decarboxylase (GAD) (synthesizes GABA) known, based on their molecular weight, the 65 and 67 kDa isoform [126]. The 67 kDa isoform is mostly expressed in perikarya and dendrites while the 65 kDa isoform is mostly expressed in axons and terminals [127]. No differences were found of GAD_{65} in schizophrenia patients compared to controls [126]. Several studies have found reduced numbers of GAD_{67} mRNA expressing neurons and overall reduced expression in schizophrenia patients compared to controls as compared to GAD_{65} [128-132]. It was found that the concentration of neurons expressing GAD_{67} was reduced in the intermediate layers of the prefrontal cortex in schizophrenia patients compared to controls, but the levels of GAD_{67} expression per neuron did not differ from controls [129]. It was proposed that 65-75% of the prefrontal cortical GABAergic neurons expressed normal levels of GAD_{67} mRNA in schizophrenia and that a subpopulation of GABAergic neurons failed to express detectable levels of mRNA [129].

GABA interneurons can be classified using three different calcium binding proteins: calretinin, calbindin and parvalbumin [126]. Calretin is mainly expressed in double bouquet and bipolar cells, calbindin 28 kDa is also mainly expressed in double bouquet cells and parvalbumin is mainly expressed in chandelier and basket cells in the prefrontal cortex [127]. Parvalbumin expressing GABA neurons have synaptic contacts concentrated on proximal axons and soma of pyramidal cells (main efferent neurons) and are therefore capable of exerting major influence on pyramidal cell firing [127]. The expression of only parvalbumin has been shown to be decreased in schizophrenia patients compared to controls [133]. The density of neurons expressing parvalbumin was not decreased in schizophrenia patients compared to controls, but the levels of parvalbumin mRNA was decreased [133]. Thus it was suggested that pyramidal cells innervating chandelier cells were selectively affected in schizophrenia [127].

Not only selective decreased expression of GAD_{67} and parvalbumin was found. Reduced expression of GABA transporter 1 (GAT1) was found in the prefrontal cortex, amygdala and hippocampus in schizophrenia patients compared to controls as assessed by ligand binding [134, 135]. However, another study failed to reproduce these data [136]. In a study using in situ hybridization no detectable GAT1 mRNA was found in a subpopulation of GABA neurons in the intermediate layers of the prefrontal cortex in schizophrenia patients

[137]. In an immunohistochemistry study, schizophrenia patients were shown to have reduced GAT1 levels in the GABAergic terminals innervating pyramidal cell proximal axons, which represent the chandelier neuron axon cartridge, compared to controls [138, 139].

The GAD_{67}, parvalbumin and GAT1 data together indicate that there is a selective GABAergic defect in schizophrenia patients. The chandelier cells innervating the proximal axons of pyramidal cells appear to be affected. Decreased expressions of GAT1 and GAD67 have opposing effects, with the former enhancing and the latter attenuating GABAergic transmission [127]. Studies regarding the composition of the GABA A receptor suggest that there is a net hypofunction of subpopulations of GABAergic interneurons in the prefrontal cortex and hippocampus [127].

Neuronal migration

The mechanism which is responsible for this localized deficit is still unknown. However, there are a few studies which could explain this phenomenon. A study which looked at the distribution of NADPH diaphorase positive neurons in the prefrontal cortex has been carried out. NADPH diaphorase is selectively expressed in a subpopulation of GABAergic interneurons [127]. It was found that schizophrenia patients had a significant different distribution compared to controls. Schizophrenia patients had a relative enrichment of NADPH diaphorase in the deeper cortical layers and a relative scarcity of NADPH diaphorase in the outer cortical layers compared to controls [140]. This is an indication of defective neuronal migration.

In the prefrontal cortex, reelin is produced by virtually every GABA neuron where reelin is secreted in the extracellular matrix and binds to dendritic spines of pyramidal neurons [141]. Reelin also appears to be involved in neuronal migration since it is expressed in Retzius cells in the fetal cortex, and can therefore be used as a marker for neuronal migration (for when neuronal migration was present during development) [127]. Reelin and reelin mRNA were found to be decreased in the (outer layers of the) prefrontal cortex, temporal cortex, hippocampus, caudate nucleus, cerebellum [142-144] and dendate gyrus [145] in schizophrenia patients compared to controls. An increased density of interstitial white matter neurons expressing less reelin was also found in schizophrenia patients compared to controls [146]. These data suggest that reelin expression is decreased in schizophrenia patients.

The reelin and NADPH diaphorase data together suggest that the localized GABA chandelier cell deficit could be due to dysfunctional neuronal migration. Migration of GABA interneuron takes place during the second trimester of pregnancy [127]. This second trimester has often been associated with an association between maternal influenza, infection, famine and an increased risk for developing schizophrenia [147].

GABA and the NMDA receptor

Like glutamate and dopamine, the GABAergic system shows deficits in schizophrenia. Unlike the other two neurotransmitters, little is known about functional changes in GABAergic transmission [123]. Moreover, it appears that the selective reduced GABA function is caused by NMDA hypofunction. GABA interneurons in the hippocampus were shown to be a 10-fold more sensitive to NMDA antagonists in schizophrenia patients compared to controls [148]. It was also shown that the NMDA receptors on GABAergic neurons were disproportionally more sensitive to a NMDA antagonist than pyramidal cells NMDA receptor [149]. These effects appeared to be restricted to limbic GABAergic interneurons [149]. Chronic treatment with an NMDA antagonist results in reduced

expression of GAD and GAT in the prefrontal cortex of the rat [150]. Furthermore, a computer model in which the NMDA component of the excitatory postsynaptic current (EPSC) of GABAergic hippocampal neurons was lost indicates that this would disrupt memory and cognitive processing in a manner similar to that found in schizophrenia patients [151]. These data indicate that the dysfunction of the GABAergic chandelier interneuron is due to NMDA hypofunction.

Recapitulation

GABAergic neurotransmission has been shown to be involved in schizophrenia. The GABAergic chandelier interneuron seems to show dysfunction, more specifically hypofunction. This dysfunction could be the result of faulty neuronal migration during the second trimester of pregnancy. It has also been shown that the dysfunctional NMDA receptor appears to be involved in the chandelier cell dysfunction. However, the exact mechanism of this GABAergic dysfunction still remains unknown.

Discussion

The above described neurotransmitters are not just independent systems; they all interact with each other. It has been shown that glutamate is involved in regulation of both dopamine and GABA. How do they interact?

The above described findings could be integrated in a single model, *figure 2*. This model is an integration of different theories, namely: mesolimbic hyperactivity, mesocortical hypoactivity, reduced thalamic inhibition from the nucleus accumbens, NMDA receptor hypofunction as a cause of schizophrenia and a cortical sensory overload as cause of the positive symptoms. However, this model also includes another theory, which is an extension of the cortical regulation of both cortical and subcortical dopamine release. This thesis shows that glutamate is involved in the regulation of dopamine transmission and it has been postulated that glutamate regulates dopamine directly through two different "systems": the "accelerator" and the "brake" [56]. The accelerator is composed of a prefrontal glutamatergic pyramidal cell and the brake is composed of a prefrontal glutamatergic pyramidal cell which projects onto a GABAergic cell [56]. Together these two systems are in balance. However, in a schizophrenia patient, with NMDA receptor hypoactivity, or when a non-schizophrenic volunteer is given ketamine, the system gets out of balance. If both are given amphetamine, dopamine is released in the prefrontal cortex, which affects mainly the brake (since the brake is responsible for reducing dopamine release); the brake fails to

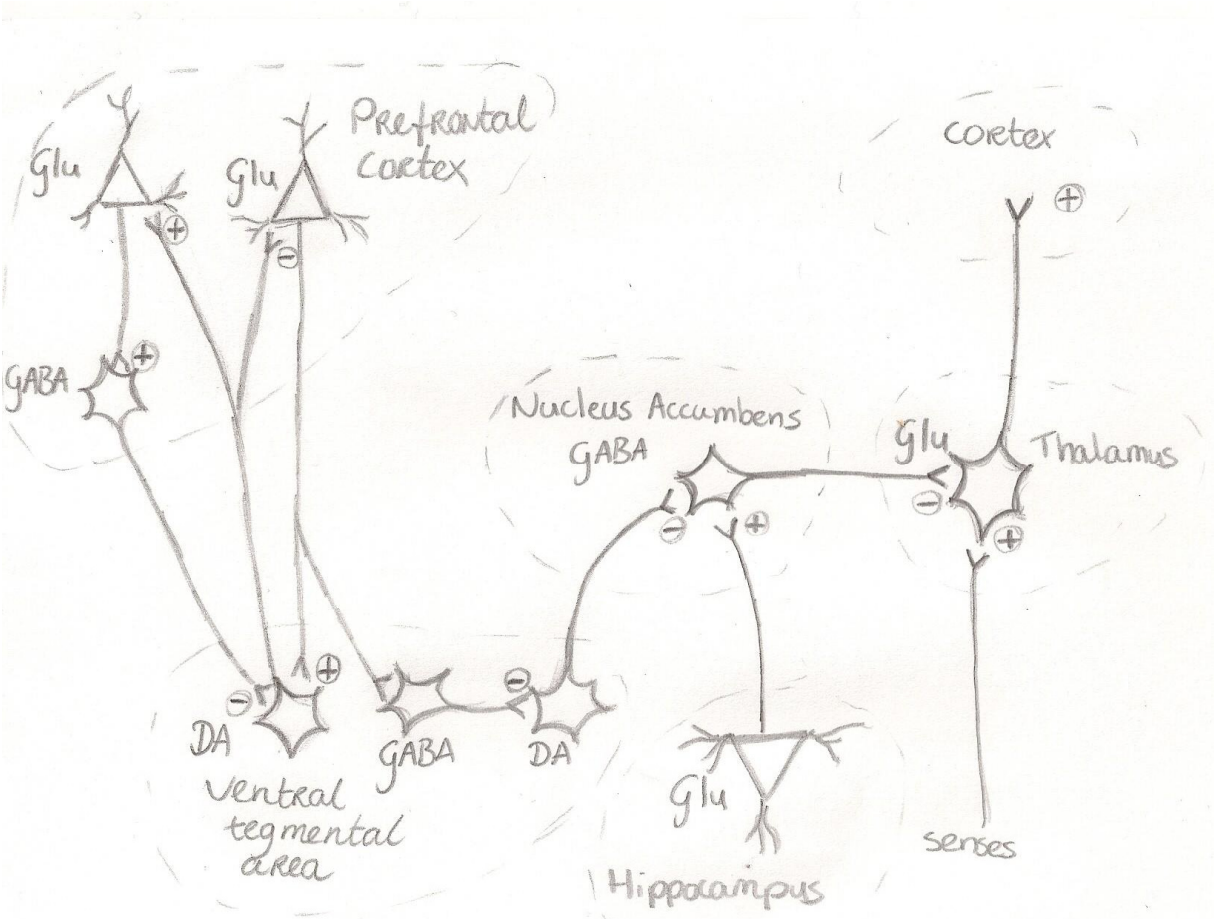

Figure 2. The neurotransmitter model of schizophrenia. The plusses reflect excitatory properties of the released neurotransmitter. The minuses reflect inhibitory properties of the released neurotransmitter. Dopamine can have its different effects through different receptors. DA=dopamine. Glu=glutamate. GABA=γ-aminobutyric-acid.

inhibit the mesocortical projection from the ventral tegmental area which leads to an enhanced dopamine release compared to a properly functioning NMDA receptor. However, since the NMDA receptor does not function in both cases, dopamine release in the prefrontal cortex is reduced compared to the normal situation since the accelerator fails to activate the mesocortical projection. This means that the accelerator/brake system can explain both the amphetamine-induced enhanced dopamine release and hypofrontality.

Besides activating, the accelerator could also do something contradictory to his name: it indirectly inhibits the mesolimbic projection. Hypofrontality was correlated to enhanced mesolimbic activity, thus the accelerator could inhibit the mesolimbic projection in its "normal" state if the accelerator projects onto a GABAergic interneuron which inhibits the mesolimbic projection. Thus, if the accelerator does not function properly due to NMDA receptor hypoactivity (or an NMDA receptor antagonist), the mesolimbic projection becomes released from the prefrontal inhibition and thus becomes hyperactive. This leads to increased dopamine in the nucleus accumbens which abolishes latent inhibition. The dopamine D_2 receptor (which is inhibitory) present in the nucleus accumbens thus reduces the activity of a GABAergic striatothalamic neuron. This same GABAergic striatothalamic neuron is activated by a hippocampal glutamatergic projection. The GABAergic striatothalamic neurons inhibit glutamatergic thalamocortical neurons which relay sensory information to the cortex. This inhibition protects the cortex from a sensory overload. Thus if the mesolimbic projection is hyperactive, the thalamocortical neurons are less inhibited which could lead to positive symptoms.

The model presented here could explain hypofrontality, amphetamine-induced enhanced dopamine release, the correlation between hypofrontality and mesolimbic hyperactivity and the correlation between mesolimbic hyperactivity and positive symptoms. However, that does not make this model the truth. For example, the model cannot explain the episodic display of symptoms.

Schizophrenia is a heterogeneous disorder, which means that schizophrenia is not caused by a single deficit [80]. That means that even though the NMDA receptor hypoactivity is involved in schizophrenia, it is not the only cause, as can be denoted from the paragraph *Genetics*. The data presented in this thesis are from schizophrenia patients. However, schizophrenia does not appear to be a single disorder, but a class of disorders [6]. Not much research has been conducted on neurobiological differences of different types of schizophrenia. Therefore, more research should be done regarding classification of schizophrenia and how these different types differ from each other in a neurobiological perspective. Therefore, even though the presented model could explain some components of schizophrenia, this might not be the case for other types of schizophrenia.

The presented model shows how dopamine, glutamate and GABA are involved in schizophrenia. However, these are not the only neurotransmitters that appear to be involved in schizophrenia. Serotonin, noradrenalin and acetylcholine have also been shown to be involved in schizophrenia, but they are less prominently involved compared to the discussed neurotransmitters [56]. The presented model can explain some part of schizophrenia and the cause of positive symptoms, although more neurotransmitters than discussed in this thesis could be involved.

To conclude, a model can be formulated that explains the positive symptoms of schizophrenia using present knowledge. However, this does not mean that this model is the

truth, since more neurotransmitters appear to be involved in schizophrenia than discussed in this thesis. I expect it to be impossible to formulate a model explaining every aspect of schizophrenia, since we simply know too little about schizophrenia. Therefore, more research has to be executed if we want to fully understand schizophrenia. Since schizophrenia is such a complex disorder, I expect this will take some time.

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