Dopamine and its interaction with DISC1 in animal models

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Dopamin I njegova interakcija sa DISC1 u životinjskim modelima

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Abstract

DISC1 is a scaffold protein described as a risk factor for major mental illnesses, including schizophrenia. It was discovered in Scottish family 23 years ago. The gene was identified at the breakpoint on chromosome 1 of the balanced translocation (1;11) (q42.1; q14.3). In the last few decades there has also been an accent on the role of dopamine in pathophysiology of mental illnesses, specifically the dopamine receptors. Since then, different hypotheses have surfaced about its involvement in those illnesses. One of the main theories is the 'Dopamine hypothesis of schizophrenia'. It described an impact of DISC1 in regulation of dopamine receptors in the striatum. DISC1 affects the receptors regulation, GSK3 pathway for D2R and KLF16 gene for D2R, and causes excessive transmission of dopamine. Increased dopamine synthesis has been related to negative symptoms of schizophrenia. For studying the pathology of DISC1 related illnesses scientists use rodent models. Their genetic, biological and behaviour characteristics closely resemble those of humans which makes them great candidates for drug development.

Key words: DISC1, dopamine, D1R, D2R

Sažetak

DISC1 je _ protein opisan kao jedan od glavnih faktora rizika u razvoju mentalnih bolesti, uključujući shizofreniju. Otkriven je prije 23 godine u jednoj škotskoj obitelji. Gen je identificiran na kromosomu 1 uravnotežene translokacije (1;11) (q42.1; q14.3). U zadnja dva desetljeća je također bio naglasak na ulozi dopamina u patozifiologiji mentalnih bolesti, točnije dopaminskih receptora. Od tada su se pojavile različite hipoteze o njegovom značaju u mentalnim bolestima. Jedna od glavnih teorija je 'Dopaminska hipoteza shizofrenije'. Ona opisuje utjecaj DISC1 proteina na regulaciju dopaminskih receptora u striatumu. DISC1 utječe na regulaciju receptora, GSK3 signalni put za D2R I KLF gen za D1R, te uzrokuje pretjeranu transmisiju dopamine. Povećano lučenje dopamine se povezuje sa negativnim simpromima shizofrenije. Za proučavanje patologije DISC1 povezanih bolesti koriste se modeli glodavca. Njihove genetske i biološke karakteristike, te karakteristike ponašanja su slične kao kod ljudi što ih čini odličnim kandidatima za razvoj lijekova.

Ključne riječi: DISC1, dopamine, D1R, D2R

Table of contents

1. Introduction

In modern world mental illnesses are becoming a rising problem affecting not only an individual fighting the disease but also its surroundings. Big part of today's population is affected by some type of mental health issue and most of them go their whole life without being diagnosed, which is why it is important to bring attention to this ongoing problem (1). Many people have mental health concerns from time to time, but when the symptoms of illness become frequent, it is needed to seek professional help. There is still a lot of stigma about these types of disorders, which is why patients aren't seeking help, even though they are aware of the problems they are facing.

The symptoms of mental illnesses are of psychological nature. For example, in schizophrenia patients those include hallucinations, extreme mood changes, confused thinking, abnormal psychomotor behaviour and other (2). If the psychiatric symptoms are not treated, they can get in a way of patients normal functioning in daily life.

As for the treatment, it depends on the type of disorder, but it is usually a combination of medications and psychotherapy. Mental illnesses consolidate a list of conditions that affect one's behaviour, mood, thinking and health in general. Some of most common examples are schizophrenia, bipolar disorder, attention deficit/hyperactivity disorder, major depressive disorder and mood disorders.

Schizophrenia is a mental disorder characterized by slow cognitive decline. It is a severe brain illness that affects 0.5%-1% of the population (3). Disrupted-in-schizophrenia 1 (DISC1) gene was shown through both animal and human studies to be the most susceptibility factor for spectrum of disorders, especially for schizophrenia. It was originally discovered in a Scottish family with high rates of schizophrenia 23 years ago, and it was identified at the breakpoint on chromosome 1 of the balanced translocation (1;11) (q42.1; q14.3) that co-segregated with mental illness (4). Since that discovery, DISC1 has been connected to numerous disorders as one of the

main triggers for the diseases. From the 77 members of the Scottish family that were genetically tested, 34 of them carried the translocation responsible for behavioural disorders. Individuals who carried the gene showcased a range of diagnoses such as major depression, schizophrenia, bipolar and emotional disorder (5).

1.1 DISC1

DISC1 is an intracellular hub protein is to regulate neurodevelopment, synapse formation, neuronal proliferation and signalling therefore it is necessary for correct brain development. Being a scaffold protein, DISC1 binds together a few other proteins, some of them also known as risk factors for major mental disorders (6). It is located around the centrosome where it interacts with other proteins. Currently there are over 200 known DISC1-interacting proteins. Some of the ones that play a crucial role together with the DISC1 are LIS1, NDE1 and NDEL1. They act as centrosomal interactors and are involved in nucleokinesis, progression of the cell cycle and dynein-related transport along microtubules (7). Other than that, the DISC1 also has a role in signalling pathways of glycogen synthase kinase 3 (GSK3), Akt pathway and the signalling that involves cyclic AMP (cAMP) messenger molecule. The exact pathway and mechanism of DISC1 is still not known which makes it a difficult drug target to this day, but also a challenge for pharmaceutical industry since it is a major risk factor for number of mental disorders.

Studies have shown that the overexpression of DISC1 in transgenic mice results in behavioural phenotypes like ones of schizophrenia and other psychological disorders (8). It was also noticed that point mutations in exons lead to the same type of behaviour. A process that occurs in the brain between early childhood and adulthood called synaptic pruning is an important step in neurodevelopment. In this key process the synapses in brain that are useless are eliminated and the ones that remain are

strengthened (9). Research has shown that DISC1 has a major role in synaptic pruning. Over pruning of those synapses has been suggested to be cause of reduction of spine density in schizophrenia patients (10).

The structure of DISC1 is still unknown. Based on the collected information so far, there are only suggestions as to what the structure would look like. A mapped secondary structure of the protein is showed in Figure 1. As for treating disorders caused by DISC1 gene, its pathway seems like a promising target for drug development (11).

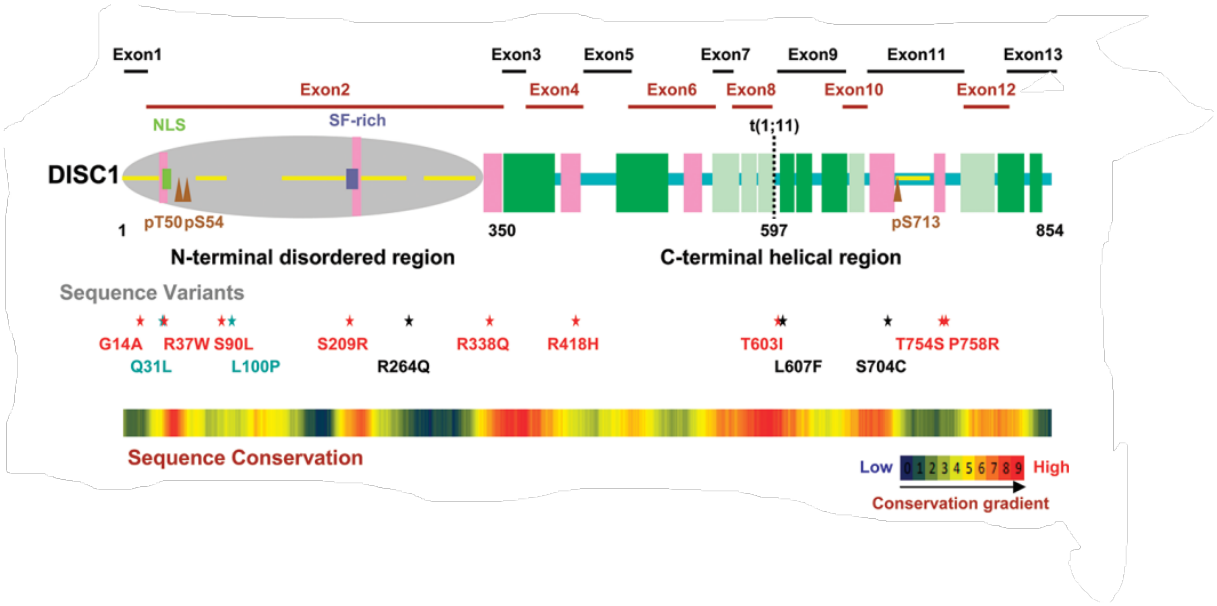

Figure 1. DISC1 schematic structure. Taken from Soares et al (6).

1.2 Dopamine

Dopamine (DA), also known as 3,4-dihydroxytyramine, is a catecholamine neurotransmitter located in human brain and it is synthesized both in peripheral and the central nervous system by the dopaminergic neurons. Typically, neurotransmitters serve as signalling molecules which are stored in vesicles in presynaptic terminal and released when stimulated (12). Catecholamine neurotransmitters are derived from amino acid tyrosine with a help of rate-limiting enzyme called tyrosine hydroxylase. When there is a need for dopamine in system, TyrH is activated to make more dopamine.

After its decarboxylation into DOPA, it is transferred to synaptic vesicles by the monoamine transporter. There, the catecholamine synthesis continues in presence of two other enzymes – dopamine-β-hydroxylase and phenylethanolamine-N-methyltransferase. Dopamine then stays in the vesicle until it is triggered by the change in extracellular levels of calcium, which then causes emptying of the vesicles (13)

Dopamine system in midbrain has two pathways, one of which controls the reward aspect of dopamine system – the mesolimbic pathway – in the ventral striatum. The other is nigrostriatal pathway which is located in the dorsal striatum and associated with motor functions (14). It controls various functions including locomotor activity, positive reinforcement, emotion, cognitive processing and sleep-wake cycle. It also contributes to synaptic plasticity in the striatum and prefrontal cortex (15). Dopaminergic system plays a pivotal role in mental disorders, which is why its dysregulation in brain leads to number of neurological and psychiatric problems. Imaging studies have shown that, for example, in schizophrenia patients, the dopamine release is significantly increased compared to the healthy controls. The increase in the levels of dopamine was also connected to signals of active psychosis (16).

2. Aims of thesis

The focus of this paper is to investigate the interaction between DISC1 and dopamine. Specifically, to explain how the DISC1 affects the dopaminergic system and its connections to psychiatric illnesses. Firstly, it will include the dopamine hypothesis from its earliest beginnings to the hypothesis we have today. The original theory initiated the whole research of dopamine and later its connection to the DISC1. Next, dopamine receptors and their role in the dopaminergic system will be explained, as well as their connection to DISC1 since they are the main characters of dopaminergic system that interact

with the protein. Last topic of the paper is going to cover animal models used for investigating mentioned interactions.

3. Dopamine hypothesis of schizophrenia

The dopamine hypothesis goes way back, up to the 1960s when it was first introduced (17). Dr. Carlsson started to investigate the role of dopamine in patients suffering from psychosis symptoms. He was significant in developing 'the dopamine hypothesis of schizophrenia' and explained the role of dopamine in the development of antipsychotic drugs (18). In the beginning the role of dopamine in schizophrenia was unknown which led to a few different hypotheses from start. The first hypothesis was based on the discovery of neuroleptic drugs. Clinical studies have shown how dopamine agonists and stimulants could worsen the symptoms of schizophrenia patients and possibly induce psychosis symptoms in healthy individuals (19). This theory was finally accepted in 1970s when there was a finding that clinical effectiveness of drug is dependent on their affinity for dopamine receptors, specifically for the dopamine D2 receptor (20).

All this data brought scientists to a theory that the cause for schizophrenia might be excessive dopaminergic transmission, also called the hyperdopaminergia. This is a state in which the levels of dopamine in the subcortex are higher than usual. The most common theory about the cause of schizophrenia is that there are too many dopamine receptors in the brain which then cause the state of hyperdopaminergia. In 1991. Davis et al have published a paper combining all the information known about dopamine role in schizophrenia up until then. In their paper they also included emerging evidence that dopamine functions could vary by brain region, specifically that dopamine 1 receptor (D1R) is characterized as predominantly cortical and dopamine 2 receptor (D2R) as predominantly subcortical (21). With this cognition they went into further research. With the animal studies they conducted, evidence showed that there is both hypo- and

hyperdopaminergia present in examined animal models. Therefore, came again an updated hypothesis that schizophrenia is characterized by frontal hypodopaminergia resulting in striatal hyperdopaminergia (Figure 2). Furthermore, it was specified that the striatal D2R hyperstimulation was the cause of positive schizophrenia symptoms, while the prefrontal cortex D1R hypostimulation caused negative symptoms. Positive symptoms include any type of change in thoughts or behaviour such as delusions or hallucinations and they are connected to the striatum since that part of the brain is responsible for decision making functions. As for the negative symptoms, they include asociality, decreased motivation, apathy and decreased emotional expression (22). Positive symptoms also tend to respond better to antipsychotic treatment then negative ones. That is why patients with more positive symptoms tend to have better prognosis and medication is more effective to them (23).

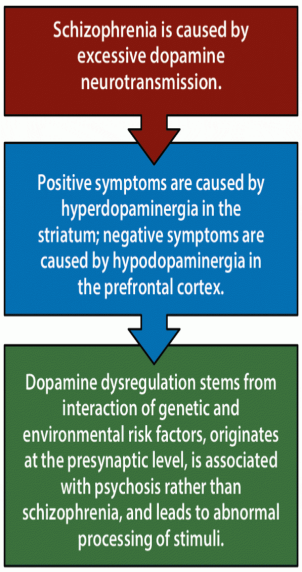

Figure 2. Dopamine hypothesis of excessive dopamine transmission. Taken from Corell et al (24).

Right now, the 'accepted' dopamine hypothesis of schizophrenia states the following – dopaminergic system dysfunction is proposed as a final pathway leading to psychosis in schizophrenia. Data from multiple genetic,

pharmacological and postmortem research studies has supported this hypothesis (25,26). The mechanism, however, by which the excess dopamine production leads to schizophrenia symptoms is still undefined (20).

It must be mentioned that the dopamine hypothesis of schizophrenia is just one of a few different hypotheses surrounding this illness. Lately, the dopamine hypothesis has been connected to the neurodevelopmental hypothesis. With merging those two theories we get a hypothesis which states that overpruning of synapses in the end causes an imbalance in excitation and inhibition of synaptic inputs to brain signalling, also known as E/I balance, from which may come the dysregulation of dopaminergic system and as the result psychotic symptoms of schizophrenia (27). Not one single gene encodes for schizophrenia, rather several genes, each of which has a small effect in making of the disease (28).

The dopamine hypothesis of schizophrenia still has room to be reworked since there is still so little that is known about the pathogenesis of this disorder. It is still missing a lot of key factors that yet have to be discovered.

4. Dopamine receptors

4.1 Dopamine receptors

Dopamine has been the topic of research for the past 30 years, as it is involved in number of pathological conditions with a major role in diseases such as schizophrenia and Parkinsons disease. It is a major neurotransmitter that regulates brain functions, cognition and neuronal activity and its system dysregulation leads to a manifestation of psychiatric conditions (29).

Dopamine has many roles in brain function. In cortex it is responsible for attention and working memory, in the basal ganglia it regulates motivational salience, reward and normal motor function and in hypothalamus it is responsible for prolactin release (30).

The effects of dopamine are regulated by dopamine receptors - members of the G-protein-coupled receptor family, also known as GPCRs. They are integral membrane proteins which are categorized as five receptor subtypes, and they are primarily divided into two larger groups based on their ligand recognition properties and their ability to modulate adenylyl cyclase (AC) - effect on cAMP production (31). Those are D1-like and D2-like dopamine receptors.

4.2 Receptor classification

In the beginning it was thought that there were only two types of receptors – D1 and D2 receptors. Afterwards it was discovered that more variants exist. It was noticed how they shared some pharmacological and pathological similarities, like a high level of homology of their transmembrane domains (29). Based on those characteristics they were divided into two larger groups. Those groups are D1-like and D2-like receptors, named after the first two known receptors. D1 receptor group includes D1R and D5R subtypes which integrate with a stimulatory G protein - G_s. On the other hand, the D2 receptor group includes D2R, D3R and D4R which couple to the inhibitory G protein $G_{i/o}$ (32).

Dopamine receptors are primarily expressed in central nervous system with D1 and D2 receptors being the most abundant ones. D1, D4 and D5 receptors are located at the post-synaptic dopamine-mediated cells, whereas the D2R and D3R can be localized both pre- and postsynaptically (33). Studies have also shown that D2-like family of receptors has a higher binding affinity to dopamine, from 10- to 100-fold higher than the D1-like

receptors. In general, D1R has the lowest affinity out of all of them (12). D1R is highly concentrated in striatum, nucleus accumbens and frontal cortex, while the D2R is mostly found in substantia nigra, hypothalamus, amygdala and hippocampus (33).

From the five receptors, the most abundant ones in the central nervous system are D1R and D2R, especially in the prefrontal cortex and basal ganglia. When it comes to genetic structure, D1 and D2 receptors differ primarily in the presence of introns in their coding sequences. The genes of D1 group of dopamine receptors, meaning D1 and D5 receptors, do not contain any introns in their coding regions. The D2 group does have several introns in receptors genetic structure. Specifically, D2 dopamine receptor encoding gene contains six introns, five are found in the gene for D3 receptor and three for the D4 dopamine receptor (29). This kind of genetic organisation in D2 receptor family allows it to be generated by receptor splice variants.

D1R and D5R receptors share around 80% of the same transmembrane structure. As for the D2-like receptors, D2R and D3R share around 75%of the transmembrane structure and the D2R and D4R around 53% (33). Both receptor types are usually segregated in striatal GABAergic medium spiney neurons (34). Once they are transferred to nucleus accumbens they work in harmony (35).

D2 receptors are G-protein coupled receptors and they exist as two isoforms – $D2_s$ (D2 short) and $D2_l$ (D2 long), which are generated by alternative splicing. As their names suggest, they differ in the length of amino acids (AA), specifically in 29 AA in third loop on $D2_l$ (36). There is still very little information about those two types of D2 receptors, but current information doesn't imply that there are any differences between them. Both reviled same pharmacological properties and the same distribution path (37,38). The splice variants of D3 and D4 receptors were also discovered, but they are still very poorly characterized (39,40).

D2 dopamine receptors are also active in non-G-protein pathways like Akt, GSK3, β-arrestin 2 and phosphatase 2A (41).

Current antipsychotics target primarily D2 receptors, but in most patients, they are ineffective and cause several serious side effects including extrapyramidal symptoms, tardive dyskinesia, sexual dysfunction, diabetes and weight gain. That is why better understanding of the details of dopamine receptors and their pathways is important for discovering new targets for therapeutics and new antipsychotics with less side effects.

One of the novel hypotheses around D2 receptor and APDs was that drugs who had agonist properties could possibly decrease dopamine transmission and have dopamine antagonist-like effects (42). The drug that currently best fits into this description is aripiprazole, a low intrinsic activity partial D2 agonist. It does not fit all the characteristics of a selective presynaptic D2 agonist, but it does come the closest to it. Positive symptoms of schizophrenia are caused by excess transmission of dopamine in mesolimbic areas, and the agonist properties of aripiprazole compete with dopamine and cause partial antagonism, which is beneficial. But in cases when the dopamine levels are low, partial agonists could work in a way that they occupy additional receptors and cause partial activation (32). The most common role of D2 receptors is signalling through adenylate cyclase, but the receptors are also involved in other pathways which include ion channels, phospholipases and MAP kinases (32).

Dopamine receptors are part of numerous psychological processes. The most studied of their roles is the locomotor activity. Evidence suggests that it is primarily controlled by D1, D2 and D3 receptors. Since D2 and D3 receptors are expressed both pre- and postsynatically, their roles are much more complex (29). D1 and D2 seem to have a main role in locomotor control, their activation is necessary for a full manifestation of locomotor activity. The D3R exhibits intermediate inhibition of the activity and D4R and D5R have minimal control of movement (29). Many vital functions also depend on the activation of dopamine receptors. As it was the case with the

locomotor activity, D1 and D2 receptors again have the most important role out of all the dopamine receptors. D3 also has a major role, but to a lesser extent. D1R and D2R are also critical actors of reward-mediated learning in the striatum.

4.3 Dopamine signalling

Dopamine signals through its receptors to induce and regulate cellular responses as well as dopamine-related behaviour. As mentioned before, D1-like receptors couple to $G\alpha_{s/olf}$ proteins and together they stimulate cAMP, which in turn stimulates activity of protein kinase A (PKA). PKA is responsible for phosphorylation of specific proteins. D2-like receptors have an opposite effect on the cAMP/PAK signalling pathway. They couple with $G_{i/o}$ protein and this complex inhibits cAMP activity which consequently lowers PKA activity (43).

4.4 Dopamine synthesis and transmission

Dopamine metabolic route involves a two-step synthesis in cytosol. Firstly, tyrosine is hydroxylated to a dopamine precursor called L-DOPA or L-3,4-dihydroxyphenylalanin. The second step is its decarboxylation to dopamine (33). This biosynthesis happens in various central and peripheral tissues. Dopamine is then transported from cytosol to synaptic vessels with the help of vesicular monoamine transporter (VMAT2). It is stored insides the vessels until release induced by nerve stell simulation. The vesicles then merge with the cytoplasmic membrane to release dopamine by exocytosis (43). Main role in dopamine degradation pathway has monoamine oxidase (MAO) which is present in outer mitochondrial membrane (12).

Extracellular dopamine levels are regulated by two main mechanisms – phasic and tonic dopamine transmission. Phasic transmission is triggered by fast action potential that activate the neuron synapse. Tonic transmission

is slower than the phasic one. It is regulated by activity of reuptake or degradation of other neurons and neurotransmitters (12).

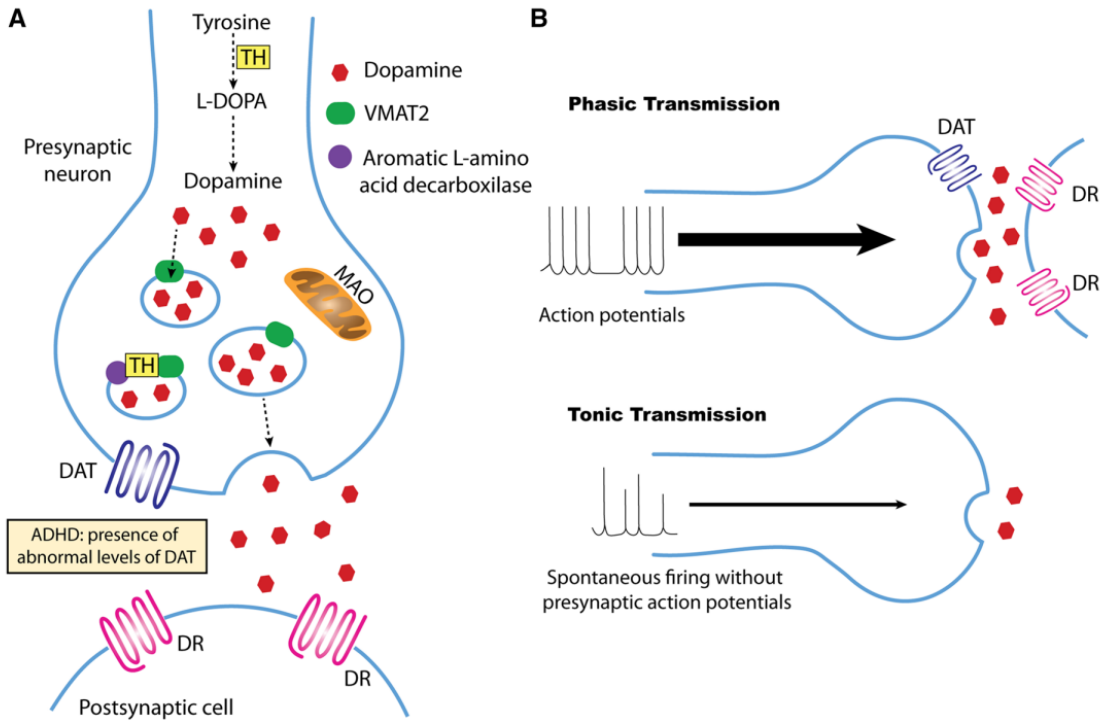

Figure _. Dopamine synthesis and transmission. Taken from Klein et al (12)

There are four dopamine signalling pathways in brain – mesocortical, mesolimbic, nigrostriatal and tuberoinfundibular pathway (44). Each pathway is responsible for a specific function in brain. Depending on what pathway is dysfunctional, different types of illnesses will develop. For example, mesolimbic pathway is in the control of emotions and reward. Therefore, if there is a dysregulation in its dopaminergic pathway there is a high chance of development of schizophrenia (33).

4.5 Structure of dopamine receptors

Dopamine receptors are as mentioned members of the seven transmembrane (TM) domain G-protein coupled receptor family and they all share most of the structural characteristics (Fig. 1). D1 and D5 dopamine receptors share 80% of their structural characteristics in their TM domains. Receptors D2 and D3 share around 75%, and D2 and D4 share about 53% identity in the structural domains. One of the greater differences in D1 and D2 receptor families is in the COOH tail, which is longer for the D2 receptor family for about seven times. In all of G protein receptors, in dopamine receptors as well, there are two cysteine residues in loops 2 and 3, which together create a disulfide bridge to stabilize the structure. But again, the two dopamine receptor families differentiate in the third loop. D2-like receptors have a long third intercellular loop which is common for receptors that interact with G_i proteins and inhibit the adenylate cyclase (AC). Whereas the D1-like receptors that interact with G_s protein are characterized by a short third loop. They also differ in the number of glycosylation spots present in their structure – D1 and D5 have two of those sites, one of which is in the extracellular loop and the other is on the NH2 terminal end. As for the second family of receptors, D2 has four possible glycosylation sites, D3 has three and D4 has only one (37).

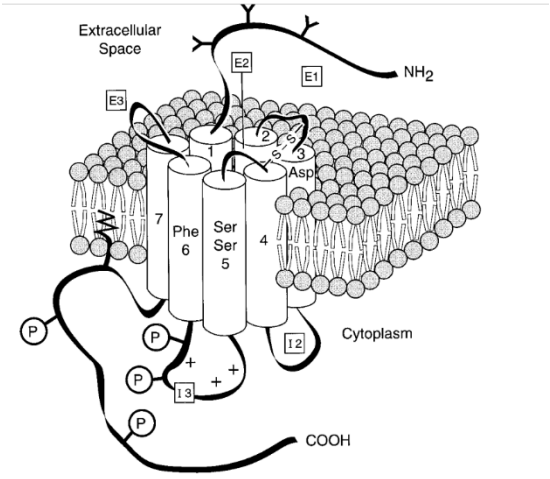

Figure 4. Dopamine receptor structures. Structural features of D1 dopamine receptor are represented. D2-like receptors are characterized by shorter COOH tail. Taken from Missale et al (37).

4.6 Dopamine receptors as drug targets

All dopamine antagonist antipsychotics share a mechanism of reducing positive symptoms of illnesses. They induct depolarization blockade at the dopamine neurons that give rise to the mesolimbic dopamine pathway. With blocking of dopamine receptors, dopamine antagonists slow down the activation of certain kind of cells (45). Understanding the function and localization of dopamine and its receptors in the brain and the complexity of their signalling mechanisms may have potential new applications in the pathogenesis of mental illnesses.

5. DISC1 and dopamine in animal models

5.1 Animal models

Studying any type of illness, whether it is of physiological or psychological nature, is not easy. One of the most important steps of any kind of research is to have a model on which the study can be conducted. There is a minimal amount of information which can be collected from patients suffering from researched disease without causing any additional problems to their current state. Testing on humans is neither practical nor ethical in most cases. As for the postmortem analyses, they are a good way of studying the illnesses. Unfortunately, not good enough because there are still some ethical problems with this approach as well as the inability to track or test the disease as it would be done in a living organism. With everything summarised, there are two main reasons for studying mental illnesses on animal models. First one is that with inducing a disease in a model, we can interrogate the neuronal mechanisms in a much more detailed way then we would in a patient or with postmortem analyses. The second one is to use it for evaluation of new developed treatment or drug (46). This is why

animal models have been introduced and to this day stay as the best available solution for studying the illnesses.

Mostly used animal models for studying of mental disorders are usually rodents. They have been proven to share the most similarities regarding genetic and brain structure, but also the behavioural phenotype. They are cost- and time-effective and more ethical to be used for testing compared to some other animal models, for example primates (47). Even though some disagree about rodents being good models for psychiatric illnesses, they are the most used in test and provide the most accurate results.

DISC1 is, as mentioned before, a scaffold protein and a major risk factor for numerous psychiatric illnesses. Together with its interacting protein it plays a role in neurodevelopment and synaptic regulation. Based on this information a hypothesis occurred, that DISC1 is in fact involved in pathophysiology of mental disorders. To test this theory, numerous rodent models with some type of DISC1 dysfunctions have been developed. Mouse DISC1 gene has been proven to be only around 60% identical to the human DISC1 (48). A lot of different types of rodent models exist in the area of research of psychiatric disorders. Since no single model can represent a disease nor are the causes and symptoms of one disease individual, there is a need for multiple models (48).

5.1.1 Knockout models

Knockout mice are models who have a gene of interest, DISC1 in this case, either disrupted or inactivated. Results from the tests done on DISC1-deficient mice showed increased levels of D1R in the striatum. DISC1 interacts with Krüppel-like factor 16 (KLF16), a transcription factor. Their interaction causes DISC1 to translocate into the nucleus at the D1R locus where it forms a complex with SIN3A protein. The result was upregulation of D1R mRNA in striatal region of DISC1-deficient mice compared to the wild type ones (Figure 3.). Collectively, these results indicate that KLF16

regulates D1r transcription by recruiting the SIN3A corepressor complex with DISC1 to the D1r promoter region (35). Dysregulation in D1 receptor functioning therefore causes cognitive impairments and exhibition of negative symptoms of the disease.

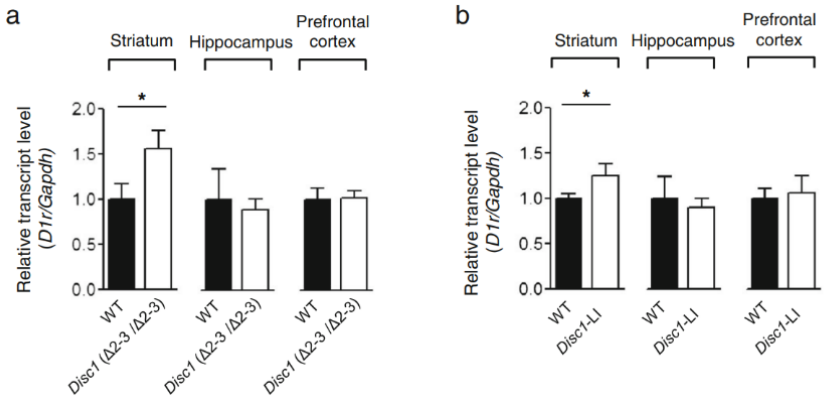

Figure 3. Upregulated transcription of D1R in DISC1-deficient mice. Taken from Suh et al (49)

5.1.2 Spontaneous mutation in the 129 strain

In the process of screening in mice models, a mutation of DISC1 gene was found. It was a 25-bp deletion in exon 6 of the 129S6/SvEv mDisc1 allele. This mutation, called $129DISC1^{Del}$, induces a frameshift in reading frame resulting in 13 new AA and a premature stop codon in exon 7 causing a termination in production of full-length protein which consequently results in specific deficits in working memory (50). To make it more like human DISC1 mutation, an additional stop codon was added at exon 8 which resulted in working memory deficits (51). It was also reported that this 25-bp mutation naturally occurs in all 129 mouse substrains (52). To date however, these effects have not been proven to be dopamine related.

5.1.3 Point mutations

This is a mutation found in exon 2 of DISC1 gene. There are two types of mutations that occur at the second exon – those are Q31L and L100P. Each

of the mutants displays specific behaviour phenotypes so it was proposed that those of Q31L are depression-like and those of L100P are schizophrenia-like (48). Mutant models exhibited deficits in working memory, reduced brain volume and decreased prepulse inhibition in conducted tests (53).

The main mechanism for regulating D2R is kinase-dependent desensitisation of receptors, endosomal trafficking and endocytosis (31). As a scaffold protein, DISC1 interacts with a number of signalling molecules to facilitate neural outgrowth, including GSK3. It was reported, from studies done on DISC1-L100P mice models, that D2Rs and DISC1 in fact form a protein complex that facilitates D2R- mediated GSK3 signalling (32). From the tests done by coimmunoprecipitation on DISC1-deficient mice models and postpartum striatum samples of schizophrenia patients, it was found that the levels of DISC1-D2R complex were elevated (54). Same thing was proven when they tested peripheral blood of schizophrenia patients (41). Levels of this protein complex were higher than normal combined with a decreased GSK3 phosphorylation. If an enhanced DISC1-D2R complex formation leads to greater GSK-3 activity and consequently to development of schizophrenia, then disrupting that complex should have antipsychotic effects and lead to a better disease prognosis. They detected a region of complex that was essential for its formation and synthesised a peptide that would break down that bond (54). Disrupting the complex successfully reversed abnormal behaviours relevant to schizophrenia in model mice. With these discoveries, some novel potential drug targets for the antipsychotic treatment of schizophrenia were proposed (41).

5.1.4 Models that mimic the Scottish translocation

The first heterozygous genetically engineered mutant DISC1 mice carrying a Disc1 allele was $DISC1^{tm1Kara}$. It is a mouse model that targets DISC1 in a way that it mimics the effects of Scottish translocation. These mice carry a

shortened lesion that stop expression of DISC1 isoforms and cause low expression of protein (55). This results in a specific deficit in spatial working memory (56). Same as with the 129 strain mice models, there are no currently known research papers that were written on the topic of $DISC1^{tm1Kara}$ and dopamine or its receptors.

5.1.5 Models with overexpressed DISC1

Over the years, animal models with overexpressed human DISC1 gene have been generated. DISC1 overexpression alone induced anxiogenic behaviour and an improvement in sensorimotor gating. It was also shown that this type of model had significant gene and environmental interactions. DISC1 protein has been shown to influence the D2R-mediated pathway in the striatum, by interacting with glycogen synthase kinase-3, as well as with the dopamine D2 receptor itself. It resulted in elevated dopamine transporter levels and elevated portions of D2 high receptor, a high-affinity form of D2 receptor, in the dorsal striatum, suggesting an altered dopaminergic state in those animals (57).

5.2 DISC1 and cilia cells

DISC1 has also been connected to the primary cilia cells in some animal studies. Cilia are known to be a specialised signalling domain of the plasma membrane where a great concentration of signalling receptors and mediators are located. Both dopamine receptors were found in high concentrations in cilia regions, so the potential link between DISC1 and the receptors was tested. The results have identified a role of DISC1 in formation of primary cilia and establish specific targeting of dopamine receptors. Exact mechanism hasn’t been discovered, but it gave scientists a new approach to studying of DISC1 and dopamine receptor interactions (58).

5.3 Validity of animal models

Animal models of neuropsychiatric illnesses are genetically modified, selectively breaded and manipulated to fit a disease profile as closely as possible. This is done based on the existing knowledge about diseases mechanisms, risk factors (from genetic ones to the environmental) and pathology in general. As said before, our understanding of psychiatric illnesses is still very minimal and that shows in the quality of produced animal models. They are not specific enough to replicate neither symptoms nor the mechanisms in the way they are expected to. There are however a few advantages of animal models over clinical cases, like that the environment can be more strictly controlled, data is easier to interpret, and groups are genetically more homogeneous (59).

An important part of developing an animal model for disease research is validity of said models. Validity can be defined as a degree to which an animal model represents the quality of tests that have been conducted. It is a fundamental feature of any research that a number of published papers unfortunately do not follow. Therefore, scientists have tried to establish some important steps in validating an animal model. They consist of three types of validators: construct, face and predictive validity (60). Construct validity refers to the extent to which the chosen method or test accurately evaluates what it is supposed to. Face validity is assessing whether the test measures or determines the thing that is supposed to. Lastly, predictive validity, refers to the ability of test to predict the future outcome. However, it must be noted that there is not such animal model that can be valid in all situations, for all research purposes. Validity is quite restrictive when it comes to uses of a model. It has to be of a specific purpose and open to debate and re-evaluation (61).

When producing an animal model, it is important to look after its validity, but it is also important to watch out for other factors that can have an impact on the results of studies, as listed in Figure 3.

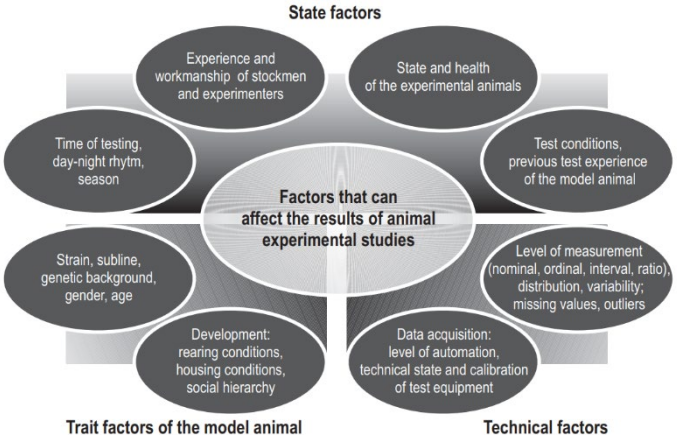

Figure 5. Factors affecting the results of animal experimental studies. Taken from Staay et al (61).

However, animal models aren't perfect and encounter quite a lot of limitations, especially ones of psychological nature. The thing with animal models is that the psychological symptoms of the diseases are either hard to distinguish or even impossible. For example, symptoms like hallucinations and delusions are not something we can register in animals like we can in patients. On the other hand, changes in social behaviour, memory and motivation can be noticed. Also, proper animal models are difficult to work with because of the complexity of human brain in comparison to a model animal brain, meaning they cannot mirror the disease effects to a full extent. It is not likely that a rodent model will mimic all the behavioural characteristics that can be seen in patients suffering from the disease nor that it would be able to precisely model just one of those behaviours (62). There are a lot of species-specific behaviour that cannot be replicated.

5.4 Gender effects on DISC1 dysfunction

Numerous genetic studies have reported sex-specific relation to psychiatric diseases. The difference can be seen in symptoms, onset disease and of course the treatment outcome. For instance, schizophrenia, shows some of those gender differences. Prevalence of this disorder is significantly higher in males, compared to females (63). They also develop the symptoms earlier on in life and the symptoms are known to be more severe. Most of the studies conducted regarding the DISC1 dysfunction have been done on male animal models, so there is still minimal information about said differences and gender effects on psychiatric disorders (64).

6. Conclusion

Since the discovery of DISC1 gene mutation in Scottish family, it has been presented as a major risk factor for mental illnesses. Numerous hypotheses about its involvement in psychiatric diseases have surfaced. The original dopamine hypothesis of schizophrenia has had a few variations over the years. The theory tries to explain the role of dopamine and DISC1 in pathology of mental illnesses, such as schizophrenia. Current hypothesis states that an imbalance of dopamine in the striatum is responsible for the development of schizophrenic symptoms, specifically the negatives ones. This imbalance is caused by dysregulation of dopamine receptors D1R and D2R.

Studies done on both postmortem brain of schizophrenia patients and DISC1-deficient mice models have showed that there is a link between the dysregulation of dopamine system and DISC1. The emphasis of these studies is on interaction of DISC1 and dopamine receptors in the striatum. The dopamine receptors are divided into two big groups – D1R and D2R – with five receptor subtypes. It was shown that DISC1 regulates pathways such as GSK3 which then consequently affects the D2R transcription. D2Rs have been studied in more detail so far, since they have shown to be a primary drug target of current antipsychotics. As for the D1Rs, their suggested way of regulation includes KLF16. This is a transcription factor in close relation with the DISC1 protein. Studies have shown that DISC1 interacts with KLF16 which then upregulates the D1R mRNA. The exact mechanisms of these processes of regulation are still unknown.

Postmortem brain analyses of patients suffering from mental illnesses have been of great significance for understanding the pathology of diseases. However, animal models have had an even bigger impact. They are the most ideal models for studying of illnesses since their genetic, biological and behaviour characteristics closely resemble those of humans, and many symptoms of human conditions can be replicated in mice and rats. They are

genetically modified to fit the symptoms and pathophysiology of studied diseases. Since no single model can project all of the symptoms and pathway dysregulations, there are a lot of subtypes of these models created each to fit a specific factor of a disease. Studies have discovered that mice have their own DISC1 variant, which is similar to around 60% to the human DISC1 variant. The mouse variant is therefore modified to represent the human one as closely as possible. Animal models are also important in drug discovery, to help understand and develop novel drugs and therapies.

In summary, the progress of understanding psychiatric illnesses in general has been very slow and the discovery of new drugs and treatments is not any better. There are a few factors that are to reason for such a slow progress – challenging neurobiology of human brain and the difficulties with examining the diseases. Without reliable models and biomarkers for studying the disease it is going to take a while for a progress to be made. With a lot of awareness being brought to mental health lately, psychiatric illnesses and their research have a potential to receive even more attention in upcoming years.

7. Literature

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