On the bumpy road of psychotic disorders

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Paving new avenues for personalized treatment approaches by examining neurochemical changes in psychosis and related disorders

Carmen van Hooijdonk

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Chapter 1

General introduction

1. Preface

This dissertation is the outcome of a four-year-long journey examining neurochemical systems in the brains of patients with psychotic disorders, as well as patients with 22q11.2 deletion syndrome (22q11DS), who are at increased risk of developing a psychotic disorder. Particularly the group of patients with psychotic disorders experience a broad spectrum of symptoms, which severely interferes with daily functioning. This dissertation aims to contribute to a better future for these individuals, by investigating neurochemical changes in the brain and their relationship with clinical symptomatology. By doing so, this work contributes to the development of a more personalized approach to treatments of psychotic and related disorders in mental health care.

2. Non-affective psychotic disorders

Approximately 1–3% of the population suffers from schizophrenia or related psychotic disorders at some point in their life.[1] These severe mental health conditions are characterized by a disconnection from reality and patients often experience a combination of positive symptoms, such as hallucinations and delusions, negative symptoms, including avolition and social withdrawal, and cognitive symptoms, such as deficits in working memory and executive functioning.[2] Within the spectrum of psychotic disorders, clinicians and researchers differentiate between affective and non-affective psychotic disorders. While affective psychotic disorders are characterized by affective dysregulation and occur simultaneously with depressive or manic episodes, this is not the case for non-affective psychotic disorders (NAPD). NAPD are the focus of this dissertation and comprise the DSM-5 diagnoses of delusional disorder, brief psychotic disorder, schizophreniform disorder, schizophrenia, schizoaffective disorder, and unspecified/other specified schizophrenia spectrum and other psychotic disorder.[3,103] In addition to NAPD, other umbrella terms, such as schizophrenia spectrum disorder, have arisen over time to describe these disorders. For instance, due to changing insights about psychotic disorders or the transfer from DSM-4 to DSM-5. All of these terms are used by different research groups and institutions and they slightly differ in their composition of DSM-5 diagnoses. As this dissertation is the result of extensive collaborations with several universities, hospitals, and mental health institutes, it uses various terms. While the scientific community has no clear preference for a particular term, we use the term NAPD in the general parts of this dissertation and explain other terms in the corresponding chapters.

NAPD are listed among the most disabling diseases worldwide.[4] In men, the typical onset of NAPD, and particularly schizophrenia, occurs in early adulthood.[5] In women, the age at onset varies more and first episodes arise in middle and even older ages.[5] Due to the often chronic course, patients frequently experience long-term

impairments in occupational and social functioning.[2] This puts a high burden on patients and their families, as well as, a high economic burden on the health care system.[6] In Europe, the total costs of psychotic disorders were estimated to be €94 billion per year in 2010.[7]

3. High-risk groups for the development of psychotic disorders

Often before the manifestation of overt psychotic symptoms, functional alterations and attenuated psychotic, negative, and cognitive symptoms are noticeable.[2] This is called the prodromal phase and clinicians often refer to this period as ultra-high risk or clinical high risk for developing a psychotic disorder. By use of operationalized criteria, such as the comprehensive assessment of at-risk mental states (CAARMS) criteria[8] or the criteria of prodromal syndromes (COPS),[9] clinicians and researchers can determine whether individuals are at clinical high risk of developing a psychotic disorder. Individuals who meet these clinical criteria have a 22% chance to transition to frank psychosis within three years after their initial presentation.[10]

Besides being at clinical high risk of developing a psychotic disorder, individuals can also be at greater risk due to genetic alterations (i.e., genetic high risk). For example, relatives of patients with a psychotic disorder have a genetic predisposition towards psychosis. Additionally, individuals with rare genetic variations, such as 22q11DS or polymorphisms in the Disrupted-in-Schizophrenia 1 protein, are at significantly increased risk for developing a psychotic disorder.[11-13] Molecular mechanisms influenced by these genetic modifications could potentially mediate the risk of developing psychosis and are described in a later paragraph of this introduction. Besides patients with NAPD, this dissertation focuses on individuals with an increased risk of developing psychosis, in particular those with 22q11DS.

4. Treatment-resistant schizophrenia

The main treatment for NAPD consists of antipsychotic drugs. These drugs antagonise dopamine D_2 receptors, which are highly expressed in the striatum, and are effective in treating positive symptoms in 67-75% of all cases.[14] Unfortunately, antipsychotic drugs are not very effective in reducing negative and cognitive symptoms, and sometimes even exacerbate these symptoms in some patients.[2] In addition, there is still a relatively large group of 25-33% of all NAPD patients, who do not respond adequately to the sequential treatment with first- and second-line antipsychotics of adequate duration, dosage, and adherence (i.e., psychotic symptoms remain).[15] These patients meet the criteria for treatment-resistant schizophrenia (TRS),[16] for which it is recommended to initiate treatment with the third-line antipsychotic clozapine. About 40% of TRS patients will respond adequately to treatment with clozapine.[14] However, currently, TRS can only be recognized retrospectively and therefore many patients with TRS are treated

with ineffective non-clozapine antipsychotic medication for a long time. In addition, they experience unnecessary, often bothersome side effects of these treatments. As clozapine treatment is also associated with several hazardous side effects, such as agranulocytosis, it would be really helpful if clinicians could predict whether their patients will respond to a particular antipsychotic drug. If so, clinicians could offer treatments with specific antipsychotics to those patients who are likely to respond to the treatment and avoid the side effects of an ineffective drug.

5. 22q11.2 deletion and duplication syndromes

22q11DS is a relatively common genetic disorder that is characterized by a microdeletion on the long arm (labelled q) of chromosome 22 at location 11.2.[17] This microdeletion occurs in minimally 1 out of 2148 live births.[18] About 85-90% of all cases have a deletion of 3 megabases in size,[19] which covers roughly 90 genes.[20] Half of these genes are protein-coding, most of which are expressed in the brain.[20] Due to the deletion, one of the two copies of the gene, normally inherited from our parents, is missing. This can result in reduced activity of the gene and reduced protein or enzyme activity (i.e., haploinsufficiency). Two of the deleted protein-coding genes are catechol-O-methyltransferase (*COMT*) and proline dehydrogenase (*PRODH*). As these genes are involved in the functioning of various neurochemical systems in the brain (described in a later paragraph of this introduction), haploinsufficiencies of these genes might affect normal brain functioning.

The phenotypic expression of 22q11DS is highly heterogeneous, hence the disorder has been known under various names in the past (e.g., DiGeorge syndrome, velocardiofacial syndrome, and Shprintzen syndrome). The phenotype of 22q11DS includes cognitive impairments (e.g., developmental delay and learning disabilities), physical problems (e.g., congenital heart disease, hypocalcemia, and palatal anomalies), as well as, mental health problems (e.g., psychotic disorders, mood disorders, and anxiety disorders).[21] More specifically, individuals with 22q11DS have a lifetime risk of developing a psychotic disorder of 20–40%,[11] which is much higher compared to the risk of 1-3% in the general population.[1] The presentation of psychotic disorders in individuals with 22q11DS is similar to that of individuals without the syndrome, with the emergence of the disorder at a similar time during development, comparable symptomatology, and similar response to antipsychotics.[22-24]

In contrast to 22q11DS, individuals with 22q11.2 duplication syndrome (22q11DUP) have an additional copy of the region of chromosome 22 that is deleted in 22q11DS. 22q11DUP has been associated with a reduced risk of developing psychotic disorders compared to the general population,[25-27] although some inconsistent results have been reported.[28] Research on 22q11DUP is currently in its infancy and only a few studies assessed neurochemical changes in this patient group.[29]

6. Precision psychiatry

Previous research has shown that longer periods between the manifestation of the first psychotic symptoms and the start of antipsychotic treatment are associated with worse outcomes with respect to quality of life and psychopathology.[30] This emphasizes the possible benefits of early intervention services, as well as, the importance of identifying individuals who are at elevated risk of developing psychosis. If clinicians can accurately predict who will transition to a frank psychosis, this offers possibilities for providing patients with personalized treatments. Currently, clinicians often use a one-size-fits-all treatment approach, in which antipsychotic medication is described by trial and error (i.e., clinicians cannot foresee which medication will work best for their patient until they tried). In contrast, precision psychiatry aims to provide each individual with the prevention or intervention strategy that she or he needs. This might be done by classifying individuals into subgroups that differ with regard to, for example, the susceptibility to a disorder, biological mechanisms, or their response to a particular treatment. In this way, prevention and intervention strategies can be offered to those who will likely benefit, while side effects of medication and healthcare costs will be spared for those who will not.

Similarly, precision oncology is a recognized form of cancer treatment, in which characteristics specific to a patient's tumour and information on the extent of the tumour's spread are used to select the best treatment option for the individual patient.[31] This approach has for instance been used for breast cancer.[32] The topic of precision psychiatry is not only relevant for the prevention of psychiatric disorders but also for the treatment of these disorders, as delays in adequate treatment with clozapine are associated with poorer clinical outcomes.[33]

To provide personalized prevention and treatment strategies for psychotic disorders, we need an advanced understanding of the neurochemical systems (e.g., dopaminergic, glutamatergic, and γ-aminobutyric acid [GABA]-ergic systems) in the brains of patients with NAPD, as well as, patients with 22q11DS or 22q11DUP. In addition, to offer individualized therapies, it is necessary to understand the association between symptom severity and molecular processes in the brain.[34] If this knowledge is available, it might be possible to optimize current treatment strategies and identify new treatment targets, which can subsequently be used to develop novel drugs that target other mechanisms than the already approved antipsychotics. This would potentially also help to effectively treat negative and cognitive symptoms in patients with NAPD. Moreover, clinicians might initiate treatment with clozapine, or other still-to-be-developed drugs, sooner in the subgroup of patients who currently do not benefit from non-clozapine antipsychotic medication.

One way of implementing precision psychiatry into psychosis care might be through the use of prediction models. These models could, based on information

provided by the patient and/or clinician, inform the prevention or intervention provider about, for example, the likelihood that an individual with 22q11DS will transition to a frank psychosis or the chance that a patient with NAPD will respond to non-clozapine antipsychotic treatment in an early stage of the illness. In this way, prediction models could support clinical decision-making in the future and contribute to individualized treatment. Prediction models can be based on several methods, one of which is machine learning. In clinical research, machine learning refers to algorithms that are capable of recognizing patterns in a large amount of multivariate data. Subsequently, these algorithms use this information to make predictions about, for instance, the clinical outcomes of an intervention for the individual patient. These predictions might be based on neuroimaging data. However, due to the complexity of NAPD, additional information, such as data about clinical and sociodemographic variables or blood markers, might be necessary to allow for the (biological) stratification of patients into subgroups (e.g., low, moderate, high risk of conversion to psychosis/response to first-line antipsychotics). Eventually, the use of prediction models might make it possible for clinicians to make more informed and patient-specific decisions about prevention and intervention strategies.

7. Investigating the neurobiology of non-affective psychotic disorders and 22q11DS

Many researchers have put effort into understanding the neurochemical systems in the brain that might be involved in the vulnerability and transition to psychotic disorders in high-risk groups, as well as, treatment response in patients with NAPD. For more than fifty years, schizophrenia research has mainly been focused on dopaminergic abnormalities. More recently, researchers have also investigated the role of other neurotransmitters, such as glutamate, GABA, and endocannabinoids.

7.1. The dopaminergic system

Neurotransmitters are chemical substances that transfer information between neurons by binding to specific receptors.[35] The neurotransmitter dopamine plays a role in various forms of behaviour, such as motor, reward-related, and cognitive behaviours. Within dopaminergic neurons, tyrosine is converted into l-3,4-dihydroxyphenylalanine (L-DOPA) by tyrosine hydroxylase. Subsequently, aromatic L-amino acid decarboxylase (AADC) converts L-DOPA to dopamine. After the synthesis of dopamine, the vesicular monoamine transporter 2 (VMAT-2) can transport and store dopamine from the cytosol into synaptic vesicles within the presynaptic synapse.[36] Dopamine that has been stored inside synaptic vesicles, can be released in the synaptic cleft (i.e., the space between presynaptic and postsynaptic neurons) via a process called exocytosis, which is influenced by action potentials.[37] After exocytosis, dopamine can bind to postsynaptic

receptors (i.e., metabotropic receptors [D_{1-5}] or dopaminergic autoreceptors [which play a role in the regulation of dopamine release]). After this interaction, extracellular dopamine can be taken up from the synaptic cleft back into the presynaptic neuron by the presynaptically located dopamine transporter.[38] Excess cytosolic dopamine can get enclosed as neuromelanin complexes inside autophagic organelles, after a process of iron-dependent oxidation, protein aggregation, and polymerization.[39]

Dopaminergic neurons mainly originate from the substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA), both midbrain regions. Dopamine travels through the brain via multiple dopaminergic pathways. The nigrostriatal dopaminergic pathway, which projects from the SNc to the dorsal striatum, is mainly implicated in the positive symptoms of NAPD,[40] while the mesocortical dopaminergic pathway, which projects from the VTA to cortical areas (mostly the frontal cortex), is mainly implicated in the cognitive symptoms of NAPD.[41]

7.2. The glutamatergic and GABAergic systems

Glutamate and GABA are the main excitatory and inhibitory neurotransmitters in the brain, respectively. Inside glutamatergic neurons, glutamine is converted to glutamate by phosphate-activated glutaminase and subsequently stored in synaptic vesicles by the vesicular glutamate transporter (VGLUT).[42,43] After the release of glutamate into the synaptic cleft, this neurotransmitter can bind to ionotropic (N-methyl-D-aspartate [NMDA], α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, and kainate) and metabotropic receptors ($mGlu_{1-8}$). In addition, glutamate can be taken up by glutamate transporters on predominantly astrocytes.[43] Within astrocytes, the astrocyte-specific enzyme glutamine synthetase reconverts glutamate into glutamine.[43] After release, glutamine can be taken up by glutamatergic neurons. Alternatively, glutamine can be taken up by GABAergic neurons, where after the conversion of glutamine to glutamate, GABA can be synthesized from glutamate by glutamic acid decarboxylase (GAD).[43] The vesicular GABA transporter stores GABA in synaptic vesicles. After release, GABA can bind to ionotropic ($GABA_A$) and metabotropic receptors ($GABA_B$), or be taken up by astrocytes, where it is metabolized to glutamate in multiple steps. This sequence of events is known as the glutamate/GABA-glutamine cycle.[43] Several researchers proposed that an imbalance between excitatory glutamatergic and inhibitory GABAergic neurotransmission might be present in some patients with NAPD (as reviewed by Wada et al. (2022)[44]).

7.3. The endocannabinoid system

The main constituents of cannabis, delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD), seem to affect the activity of the endocannabinoid system (ECS).[45,46] The ECS is an endogenous signalling system that consists of two main

endogenous endocannabinoids (i.e., N-arachidonoylethanolamine [anandamide] and 2-arachidonoylglycerol [2-AG]), their synthesizing (i.e., N-acylphosphatidylethanolamine selective phospholipase D and diacylglycerol lipase) and degrading enzymes (i.e., fatty acid amide hydrolase and monoacylglycerol lipase), and two main cannabinoid receptors (i.e., type 1 [CB_1] and type 2 [CB_2]).[47,48] Several physiological processes throughout the body are regulated by the ECS, such as inflammation,[49] sleep,[50] and cognition.[51] In the brain, several neurotransmitter systems are modulated by the ECS, for example, dopaminergic,[52] glutamatergic, and GABAergic systems.[53] As frequent cannabis use in early adolescence has been associated with an increased risk of psychosis (as reviewed by Marconi et al. (2016)[54] and Howes et al. (2004)[55]), a potential role of the ECS in the pathophysiology of psychosis has been proposed.[56] In addition, a meta-analysis reported increased concentrations of anandamide in blood and cerebrospinal fluid of patients with psychotic illnesses relative to controls.[57]

8. Imaging methods for investigating neurobiological mechanisms

Molecular imaging techniques, such as positron emission tomography (PET), single photon emission computed tomography (SPECT), neuromelanin-sensitive magnetic resonance imaging (NM-MRI), and proton magnetic resonance spectroscopy (^{1}H-MRS), have enabled the *in vivo* examination of various aspects of neurotransmitter systems in the brain.

PET and SPECT are nuclear imaging techniques, which can be used to investigate components of the dopaminergic system, such as dopamine synthesis capacity (DSC), by measuring the uptake of specific radiotracers that interact with these components. When radioactive tracers decay, photons (in the case of SPECT) and positrons (in the case of PET) are emitted. Within a few millimetres, the positrons collide with electrons present within the tissue, which causes annihilation. During annihilation, two photons (i.e., gamma rays) are emitted in opposite directions.[58] The gamma-photons, in the case of both PET and SPECT, are detected by one or more detectors that surround the subject to form images. [^{18}F]F-DOPA PET has successively been used to assess striatal DSC.[59-62] The radiotracer is moved across the blood-brain barrier, transported into dopaminergic neurons via an amine acid transporter, and subsequently decarboxylated to [^{18}F]-fluorodopamine by AADC and stored within synaptic vesicles. This method, therefore, predominantly reflects presynaptic dopamine synthesis. Often, [^{18}F]F-DOPA uptake is investigated in a region of interest relative to a reference region with low radiotracer uptake, such as the cerebellum. In addition, the high-affinity antagonist radiotracer [^{18}F]fallypride is a substituted benzamide and [^{18}F]fallypride PET has repeatedly been used to assess the availability of (extra)striatal dopamine $D_{2/3}$ receptors.[63-67] Similar to [^{18}F]F-DOPA procedures, (extra)striatal availability of dopamine $D_{2/3}$ receptors could be evaluated compared to a reference

region devoid of dopamine $D_{2/3}$ receptors, such as the cerebellum. Lastly, [^{123}I]FP-CIT is a validated SPECT tracer to investigate the presynaptically located dopamine transporter, which is found predominantly in the striatum.[68] Often the cerebellum or occipital cortex is used as a reference region when assessing specific striatal binding. Although [^{123}I]FP-CIT is not a selective radiotracer for the dopamine transporter (i.e., the tracer has also a modest affinity for the serotonin transporter),[69,70] studies in healthy individuals showed that selective serotonin reuptake inhibitors block extrastriatal, but not striatal (both relative to the cerebellum), [^{123}I]FP-CIT binding.[69] Successively, many hospitals and institutes use this tracer to assess the integrity of the nigrostriatal pathway in routine clinical practice.

^{1}H-MRS is used non-invasively to investigate the concentration of metabolites which are present in small amounts in brain tissue. Protons in water- and fat-containing tissues are the most important source of the MRI signal, but these abundant signals are excluded during ^{1}H-MRS. During ^{1}H-MRS, tissue is exposed to an external magnetic field. This causes the nuclei of atoms to resonate at a certain frequency (i.e., resonant frequency).[71] This resonant frequency depends on the strength of the external magnetic field and the local microenvironment of the atomic nucleus. The nucleus is surrounded by a cloud of electrons. In response to the external magnetic field, these electrons produce their own magnetic field (this is called shielding). This causes a change in the local magnetic field, which subsequently causes a shift in the resonant frequency. This principle is called chemical shift (δ).[72] Concentrations of different metabolites, such as glutamate and GABA, can be measured as their resonant frequencies slightly differ.[72]

NM-MRI is an MRI sequence, which is sensitive to the neuromelanin content in tissue. Neuromelanin is an insoluble, black pigment that accumulates mainly in the dopaminergic cells of the SNc.[73] Neuromelanin-iron complexes cause T1-shortening due to paramagnetic properties and magnetization transfer effects.[74,75] This creates a notable contrast in NM-MRI signal between the substantia nigra and the surrounding brain tissue.

9. Current insights in the neurobiology of non-affective psychotic disorders

The so-called dopamine hypothesis of schizophrenia proposes a framework that links the interplay between various risk factors, such as genetic disposition, stress, and drug use, to a final common pathway of presynaptic striatal hyperdopaminergia (i.e., increased synthesis and/or release of dopamine in the striatum of patients).[76] The presynaptic striatal hyperdopaminergia is thought to result in the abnormal allocation of salience to neutral stimuli, consequently resulting in the development of psychotic symptoms.[76] Many [^{18}F]F-DOPA PET studies have found increased striatal presynaptic

DSC (i.e., an indicator of hyperdopaminergia) in patients with psychosis, but not in patients with TRS (as reviewed by Brugger et al. (2020)[77]).

Alterations in dopaminergic functioning by themselves do not explain all facets of psychotic disorders. Subsequently, the involved of other neurochemicals, such as GABA and glutamate, in the pathology of psychotic disorders has been suggested. This suggestion is based on studies that showed that antagonists, such as phencyclidine (PCP) and ketamine, that block NMDA receptors which are located on GABAergic interneurons, cause schizophrenia-like symptoms to occur in healthy volunteers and exacerbate these symptoms in patients (as reviewed by Howes et al. (2015)[78]). Subsequently, the glutamate hypothesis of schizophrenia was proposed. This hypothesis suggests that hypo-functioning of NMDA receptors on cortical fast-spiking GABAergic interneurons leads to decreased GABAergic inhibition of glutamatergic pyramidal neurons, which results in an excessive release of glutamate.[79,80] Accordingly, previous *in vivo* ^{1}H-MRS studies have found higher glutamate levels in the anterior cingulate cortex (ACC) of patients with TRS compared to responders,[81-83] although higher glutamate levels might be explained by greater illness severity.[84,85] In addition, multiple meta-analyses of ^{1}H-MRS studies reported reduced GABA levels in frontal brain areas in NAPD,[86,87] which suggests that the balance between glutamatergic and GABAergic functioning might be altered in NAPD.

The glutamate and dopamine hypotheses are not mutually exclusive. The glutamate hypothesis can function as an extension of the dopamine hypothesis, and together they suggest that presynaptic striatal hyperdopaminergia might be secondary to changes in glutamatergic functioning in patients with schizophrenia.[78] Moreover, as NAPD are very heterogeneous, it has been suggested that several subgroups of patients with different neurobiology exist.[88] Specifically, as researchers reported elevated striatal DSC exclusively in patients who responded well to first- and/or second-line antipsychotic medication (i.e., responders) and not in patients with TRS compared to healthy individuals,[61,62] there might be a subgroup of patients with hyperdopaminergic functioning, while another subgroup might exhibit normodopaminergic functioning.[88]

Overall, the current insights indicate that several neurotransmitter systems are involved in the pathophysiology of NAPD. Hence, medication approaches might need to be amended to target the(se) specific system(s) that is/are altered in subgroups of patients. To optimize current treatment targets and develop novel selective drugs that target different mechanisms than the currently approved drugs, we need a more advanced understanding of the neurobiology of NAPD.

10. Current insights in the neurobiology of 22q11.2 deletion syndrome

The *COMT* and *PRODH* genes are haploinsufficient in 22q11DS. The *COMT* gene encodes the *COMT* enzyme, which plays a role in the breakdown of catecholamines,

such as extracellular dopamine. The haploinsufficiency of the *COMT* gene is thought to especially affect frontal dopamine levels,[89] due to a relative paucity of dopamine transporters in the frontal cortex.[90] Previous studies have assessed dopaminergic functioning in individuals with 22q11DS and found some evidence for presynaptic striatal hyperdopaminergia.[29,91] Moreover, the availability of dopamine $D_{2/3}$ receptors is reduced in frontal brain areas in 22q11DS,[92] as well as, in the striatum of individuals with 22q11DS who carried the methionine (low activity) allele compared to individuals who carried the valine (high activity) allele of the *COMT* gene.[93] These findings suggest that dopaminergic abnormalities might occur before high-risk individuals develop a psychotic disorder. If so, high-risk patients might benefit from early intervention strategies that target these alterations.

Besides changes in dopaminergic functioning, alterations in glutamatergic functioning might occur in 22q11DS. This has been suggested as the *PRODH* gene encodes the *PRODH* enzyme, which is involved in the degradation of proline. During this process, glutamate is produced. As proline can activate the glutamatergic NMDA receptor[94,95] and haploinsufficiency of the *PRODH* gene is associated with increased proline levels,[96] this might result in increased stimulation of NMDA receptors by proline and therefore elevated release of glutamate in 22q11DS.[94,97] Elevated glutamate and Glx (glutamate plus glutamine) concentrations have been reported in the hippocampus of individuals with 22q11DS who developed schizophrenia relative to individuals with 22q11DS who did not.[98] However, recent ^{1}H-MRS studies found no alterations in glutamatergic functioning in the striatum or ACC of individuals with 22q11DS compared to controls.[99,100]

Due to the known genetic cause of 22q11DS and the high prevalence of mental health problems (including psychosis) in this patient group, 22q11DS is a promising model for studying the pathophysiology of psychotic disorders. Therefore, obtaining a better understanding of the neurobiology of 22q11DS might result in new treatment approaches and the characterization of early indicators of neuropsychiatric disorders, which are relevant for individuals with and without 22q11DS.

11. The present thesis

The overall aim of this dissertation is to advance the current knowledge of neurobiological processes in individuals with an increased risk of developing a psychotic disorder and individuals with NAPD by using different imaging approaches. By doing so, this work contributes to the development of a more personalized approach to treatments for psychotic disorders in mental health care. This dissertation is divided into three parts.

In the first part, neurobiological mechanisms were examined in individuals at increased risk of developing a psychotic disorder, in particular 22q11DS. **Chapter two**

presents a literature overview of *in vivo* neuroimaging studies that address the dopaminergic system of individuals at increased risk of developing a psychotic disorder (among others, individuals with 22q11DS). As individuals with 22q11DS also have an increased risk of early-onset Parkinson's disease[101] and Parkinson's disease is characterized by the loss of striatal dopamine transporter binding,[102] **chapter three** examines differences in the availability of the striatal dopamine transporter between 22q11DS, 22q11DUP, and healthy volunteers by use of [^{123}I]FP-CIT SPECT. In **chapter four**, using ^{1}H-MRS and [^{18}F]fallypride PET, we continued to study neurobiological mechanisms in patients with 22q11DS and investigated the relationships between dopaminergic, glutamatergic, and cognitive functioning in these individuals.

In the second part, neurobiological mechanisms were examined in individuals with NAPD. **Chapter five** presents a literature overview of post-mortem and molecular imaging studies that address molecular alterations in the substantia nigra of patients with schizophrenia. As previously mentioned, information about dopaminergic changes, for instance, as assessed with [^{18}F]F-DOPA PET imaging, might help to predict response to antipsychotic treatment in patients with NAPD. As PET imaging is time-consuming, expensive, and associated with a high burden for patients, we would preferably make use of an alternative method such as NM-MRI. However, the interrelationships between NM-MRI and [^{18}F]F-DOPA PET measures are unknown. Therefore, in **chapter six**, using NM-MRI and [^{18}F]F-DOPA PET, we investigated the relationship between striatal DSC and neuromelanin in the substantia nigra of patients with NAPD and healthy volunteers. Lastly, besides neuroimaging markers, other more easily obtainable markers (e.g., blood markers) might be useful to stratify NAPD patients into subgroups. Hence, in **chapter seven**, we compared plasma concentrations of endocannabinoids between NAPD patients and healthy individuals. In addition, we investigated whether endocannabinoid plasma concentrations were related to dopaminergic, glutamatergic, and GABAergic functioning in both groups, as assessed with [^{18}F]F-DOPA PET and ^{1}H-MRS.

In the future, clinicians might be supported by technology that can help clinical decision-making and thereby contribute to a more personalized approach to treatment in mental health care. In the final part of this dissertation, we elaborate on this future perspective. In **chapter eight**, we assessed by use of a machine learning model and data from the Genetic Risk and Outcome in Psychosis (GROUP) study, whether clinical, familial, environmental, and sociodemographic variables, which could potentially predict TRS in the future, were associated with TRS in patients with NAPD.

Finally, the findings of this dissertation are summarized in **chapter nine** and discussed in **chapter ten**. In **chapter eleven**, a summary of the key findings is provided in Dutch.

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Part

Neurobiology of individuals with an increased risk of developing a psychotic disorder: 22q11DS

Chapter

Dopaminergic alterations in populations at increased risk for psychosis: a systematic review of imaging findings

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Abstract

Alterations of the dopaminergic system may be important neurobiological correlates of vulnerability and transition to psychosis. We systematically reviewed the evidence for dopaminergic alterations demonstrated by *in-vivo* imaging studies in humans at increased risk of developing psychosis, covering clinical, genetic, and environmental high-risk groups. All 63 included studies utilized positron emission tomography (PET), single photon emission computed tomography (SPECT), or neuromelanin-sensitive magnetic resonance imaging (NM-MRI) methods to collect data concerning the dopaminergic system during rest and/or following pharmacological, behavioural, or cognitive challenges. The current evidence highlights that 1) striatal dopamine $D_{2/3}$ receptor availability is unaltered in all three high-risk groups compared with healthy individuals; 2) striatal dopamine synthesis capacity (sDSC) is increased in some clinical and genetic high-risk individuals relative to controls (e.g., people that meet clinical criteria for being at ultra-high risk of developing psychosis and individuals with 22q11.2 deletion syndrome), while sDSC is decreased in cannabis-using environmental high-risk individuals. It seems likely that all three high-risk groups can be stratified into multiple subgroups, with varying risks to develop psychosis, transition rates, and underlying neurobiology. The present results support the hypothesis that dopaminergic abnormalities occur before high-risk individuals develop psychosis.

1. Introduction

Approximately 1–3% of the population suffers from schizophrenia and related psychotic disorders.[1] Often, identifiable symptoms and functional alterations precede the development of a first psychotic episode.[2] The longer the time between the occurrence of the first psychotic symptoms and the start of adequate treatment, the worse the subsequent improvement in psychopathology and quality of life.[3] Subsequently, Correll et al. (2018)[4] showed that early intervention services were related to superior outcomes compared with treatment as usual in first-episode psychosis. These findings highlight the potential benefits of early interventions and the importance of the identification of individuals who are at elevated risk of developing psychosis and their corresponding risk of transitioning.

High-risk individuals can be divided into clinical, genetic, and environmental high-risk groups. First of all, the clinical high-risk group comprises people that meet clinical criteria for being at ultra-high risk (UHR) of developing psychosis, according to, among others, the Comprehensive Assessment of At-Risk Mental States (CAARMS) criteria[2] or the Criteria of Prodromal Syndromes (COPS).[5] These operationalized criteria are based on the presence of attenuated psychotic symptoms, the occurrence of a brief limited intermittent psychotic episode, or a familial genetic risk and deterioration syndrome.[6] The genetic risk and deterioration syndrome group entails individuals with functional decline and a family history of psychosis or individuals with schizotypal personality disorder (SPD) with functional decline.[6] Individuals who meet the clinical criteria are designated as being at UHR of psychosis and have a 22% risk to transition into a frank psychosis within the three years following their initial clinical presentation.[7] Additionally, SPD is independently associated with an increased risk of developing schizophrenia compared with the general population.[8] The term schizotypy not only refers to patients with SPD but also relates to healthy people in the general population with schizotypal personality traits.[9] Individuals who score high on positive schizotypy (characterised by, among others, suspiciousness, unusual perceptual experiences, and odd beliefs) exhibit an elevated risk to develop affective disorders, as well as, non-affective psychotic disorders. Individuals who score high on negative schizotypy (characterized by, among others, affective flattening, social disinterest, and anhedonia) are at risk particularly for schizophrenia spectrum disorders.[10] Both of these schizotypy groups are assigned to the clinical high-risk group, as the UHR criteria developed in the early 1990s also refer to the positive features of schizotypy.[11,12] In addition, physical anhedonia, which is part of the negative features of schizotypy, has been associated with the clinical high-risk state.[11]

Secondly, the genetic high-risk group includes individuals with a genetic predisposition to schizophrenia, but without functional decline, such as relatives of schizophrenic patients. The degree of risk is associated with the degree of genetic

relatedness.[13] For example, grandchildren of schizophrenic patients have a lifetime risk of 5% to develop schizophrenia, while offspring with one parent with schizophrenia and monozygotic twins of schizophrenic patients have a risk of 17% and 48%, respectively.[13] In addition to family members of affected individuals, people with rare genetic variations can also be at increased risk of developing psychosis. For instance, schizophrenia is 20–25 times more common in individuals with 22q11.2 deletion syndrome (22q11DS).[14] This is a genetic disorder caused by the deletion of a small section of chromosome 22q11.2.[15] Other genetic alterations that have been linked to the risk for psychosis are polymorphisms in the Disrupted-in-Schizophrenia 1 (DISC1) protein.[16,17] Molecular mechanisms affected by these genetic alterations could potentially mediate the risk of developing psychosis.

Lastly, the environmental high-risk group consists of individuals who are highly exposed to environmental risk factors that have been associated with schizophrenia, such as, childhood trauma, cannabis use, stress, and migration.[18]

Many researchers have put effort into understanding the neurobiological correlates of transition and vulnerability to psychosis in these clinical, genetic, and environmental high-risk groups. If modifiable correlates exist, this would potentially accommodate personalized therapeutic/preventive strategies. By predicting who will go on to develop psychosis and by offering starting points for the development of new early interventions, the subsequent transition of high-risk individuals to the first psychotic episode may be avoided.

One neurobiological correlate of particular interest has been the neurotransmitter dopamine (DA), whereas striatal DA dysfunction has been the leading theory for the pathophysiology of schizophrenia.[19] Molecular imaging techniques, such as positron emission tomography (PET), single photon emission computed tomography (SPECT), and the more recently developed neuromelanin-sensitive magnetic resonance imaging (NM-MRI), have enabled the *in vivo* investigation of multiple components of the dopaminergic system. For example, researchers have studied the striatal dopamine synthesis capacity (sDSC), availabilities of the DA $D_{2/3}$ receptor, DA D_1-like receptor and dopamine transporter (DAT), and psychosocial stress- or pharmacologically-induced DA release. An overview of the PET, SPECT, and NM-MRI molecular imaging techniques and their corresponding targets is presented in eTable 1.

A recently published meta-analysis by Brugger et al. (2020)[20] demonstrated that elevated sDSC and DA release capacities may be core features of schizophrenia, while changes in DAT and DA $D_{2/3}$ receptor availabilities or synaptic DA concentrations may only occur in subgroups of patients. In addition to the DA alterations observed in schizophrenic patients, alterations in the dopaminergic system of individuals of the clinical, genetic, and environmental high-risk groups have also been reported: an

elevated sDSC has been found in UHR individuals,[21] first-degree relatives of schizophrenic patients,[22] individuals with 22q11DS,[23] and has been associated with environmental risk factors for psychosis such as childhood adversity.[24]

This systematic review aims to summarize the results of PET, SPECT, and NM-MRI imaging studies, that address different parts of the dopaminergic system in populations with an increased risk of developing psychosis (i.e., clinical, genetic, and environmental high-risk groups) and, if available, compare these findings to the dopaminergic system of healthy controls (HC) and schizophrenic patients. We will evaluate original studies that investigated the dopaminergic system in rest (i.e., at baseline conditions) or before and after a challenged state. This challenged state can be produced, by pharmacological, behavioural, or cognitive means, to obtain insight into dynamic changes of the dopaminergic system.

Recognizing individuals with an increased risk for developing psychosis and predicting who most likely will develop a frank psychosis, creates opportunities for early interventions. This could slow down or even prevent the onset of psychosis. Indeed, a recent study showed that 36.9% of the proportion of clinical psychosis outcomes could have been avoided if the psychosis high-risk state had been prevented.[25] Essential for the prediction of transition to psychosis and the development of urgently needed early interventions is an understanding of the underlying neurobiological correlates of vulnerability to psychosis.

2. Methods

This systematic review was conducted in accordance with the Preferred Reporting Items For Systematic Reviews and Meta-analyses (PRISMA) reporting guideline.[26] The review protocol was registered in the international prospective register of systematic reviews (PROSPERO; CRD42020173412).

2.1. Search strategy, selection criteria, and data extraction

The search for published studies was conducted from inception to April 20, 2021, in PubMed and PsycINFO (see eMethods 1 for the search strategy). Furthermore, reference lists of relevant meta-analyses, reviews, and included original articles were hand-searched to identify missing studies. All titles and abstracts of retrieved publications were independently screened by two researchers (CvH [author] and DK [student assistant, see acknowledgements]) to assess eligibility for inclusion. If necessary, the full text of the article was reviewed and disagreements between the two researchers were solved by consensus. After the initial screening, both researchers (CvH and DK) independently screened the full-text versions of the initially selected studies to assess the inclusion and exclusion criteria. Inclusion criteria were original articles, published in the English language, which reported brain imaging data of the

dopaminergic system in participants at high risk for developing psychosis. Animal studies, duplicate publications, or studies that reported data of at-risk participants with DA dysregulation or psychotic symptoms in the context of neurological disorders (e.g., Parkinson's disease or epilepsy) were excluded. All studies that did not meet the inclusion criteria or met the exclusion criteria during the full-text screening were excluded. The reasons for exclusion are documented in eTable 2. In the case of disagreements between the two researchers responsible for screening, TvA helped to resolve the discrepancies. Both researchers (CvH and DK) individually extracted data from all included studies into an electronic summary table.

2.2. Outcomes

We investigated the dopaminergic system of at-risk populations by reviewing the data of PET, SPECT, and NM-MRI studies. At-risk populations included individuals at clinical high risk (e.g., due to the presence of transient psychotic symptoms), genetic high risk (e.g., familial or other genetic predisposition to develop psychosis), and environmental high risk (e.g., individuals exposed to environmental risk factors associated with psychosis). The DA imaging data consisted of data collected during rest (i.e., at baseline conditions), as well as, data collected following pharmacological, behavioural, or cognitive challenges. The results in this systematic review, therefore, concern various aspects of the dopaminergic system, namely: sDSC, DAT availability, DA D_1-like and DA $D_{2/3}$ receptor availabilities, synaptic DA and neuromelanin concentrations, and psychosocial stress-, pharmacologically- or cognitive task-induced DA release. If available, the results were compared with the dopaminergic functioning of HC and, if presented in the same article, schizophrenic patients.

2.3. Quality assessment of the included studies

The case-control and cross-sectional versions of the Observational Study Quality Evaluation (OSQE) were used for the risk of bias assessment of observational studies.[27] This tool was developed while the existing risk of bias assessment tools have important disadvantages. The OSQE combines criteria from three different criteria lists: the Newcastle-Ottawa Scale (NOS),[28] the Strengthening the Reporting of Observational Studies in Epidemiology (Strobe),[29] and the Critical Appraisal of a Topic criteria list used in educational programs at Maastricht University, the Netherlands.[30-32] Before the OSQE can be used to assess the quality of the included articles, an information sheet needs to be completed to adjust the OSQE to the research question of our interest (see eMethods 2). Subsequently, fifteen (in case of case-control study designs) and eight items (in case of cross-sectional study designs) evaluate, amongst others, the representativeness of the study sample, the assessment of the independent and dependent variables, the amount of non-response, and the statistical adjustment for

confounders in each included study. For each item, a star can be appointed. Two researchers (CvH and MD/DK/AW [see acknowledgements]) independently rated the quality of each study. Disagreements in ratings were discussed between the researchers, which resulted in final consensus ratings.

3. Results

The study selection procedure is summarized in the PRISMA flowchart (Figure 1).[26] Sixty-one studies, retrieved from the PUBMED and PsycINFO literature search, met the above-mentioned criteria (see Section 2.1). Additionally, one unpublished manuscript was included and one article was included after screening the references lists of relevant reviews and included articles. The included studies used different methodologies to investigate the dopaminergic system during rest conditions and/or following a pharmaceutical, behavioural, or cognitive challenge. This resulted in 33 studies on DA $D_{2/3}$ receptor availability, 15 studies on sDSC, 12 studies on psychosocial stress-induced DA release, 15 studies on pharmacologically-induced DA release, 3 studies on cognitive task-induced DA release, 3 studies on DA D_1-like receptor availability, 2 studies on DAT availability, and 1 study on neuromelanin concentrations (see Table 1). In the following part of the review, the different parts of the dopaminergic system will be discussed per high-risk group.

3.1. Clinical high-risk groups

3.1.1. UHR individuals

Fifteen studies reported on DA imaging data in UHR subjects.

3.1.1.1. sDSC

Five studies compared the sDSC in the whole striatum (WS) and its functional subdivisions, i.e., associative (AST), limbic (LST), and sensorimotor subdivisions (SMST), between UHR and HC subjects.[21,33-36] Two robust findings were the absence of sDSC group differences in the SMST and LST. However, the results were less consistent with regard to the WS and AST. No differences in the WS[36] and AST[33,36] were reported, as well as, elevations in the WS[21,34] and the AST.[21,34,35] UHR subjects did not differ from schizophrenic patients with regard to sDSC in the WS or any of its functional subdivisions.[21] Interestingly, Howes et al. (2009)[21] reported positive correlations between sDSC in the WS, AST, and SMST and the severity of psychotic symptoms as indexed by total CAARMS and Positive and Negative Syndrome Scale (PANSS) scores in UHR subjects. However, these findings were not replicated in a second cohort.[34] Some of the previously described studies re-invited the UHR subjects for a follow-up assessment and compared the sDSC between UHR individuals who in the meantime had transitioned to a first-episode psychosis (i.e., UHR transition group)

and those who had not developed a frank psychosis (i.e., UHR non-transition group). Elevations of sDSC have been found in the WS[8] (follow-up time was at least three years) and AST[8,33] (Allen et al. (2012)[33] did not specify the length of their follow-up assessment) of the UHR transition group compared with the UHR non-transition group, along with no group differences in the LST[8,33] and SMST.[8] However, a subsequent study by Howes et al. (2020)[36] (median follow-up length was fifteen months; PET data was not previously reported) did not find any baseline sDSC differences between the UHR transition and non-transition subjects in the WS or AST (Howes, personal communication). Nonetheless, elevated sDSC in the WS and AST predicted a worsening of psychotic-like symptoms at follow-up.[36] An elevation in sDSC in the WS and AST was also found in UHR transition individuals relative to HC.[8] No sDSC group differences were reported between the UHR transition group and HC in the SMST and LST.[8] Furthermore, UHR non-transition subjects did not differ from HC in sDSC in the WS or one of the striatal subdivisions.[8] Within transitioned UHR subjects, there was a positive association between sDSC in WS and total CAARMS and PANSS scores.[8] No such association was evident in the UHR non-transition group.[8] In conclusion, multiple studies, from the same research group, hint towards increased sDSC in the WS and AST of UHR individuals compared with HC, especially in those subjects who subsequently developed a first-episode psychosis. However, not all studies support these findings.

3.1.1.2. Neuromelanin

Neuromelanin is a product of the metabolism of DA and has recently been suggested as a non-invasive proxy measure of dopaminergic functioning,[37] which can be determined by MRI. Cassidy et al. (2019)[37] found no differences in NM-MRI signal (i.e., the tissue concentration of neuromelanin) between UHR and HC or between UHR and schizophrenic patients in the substantia nigra (SN). However, UHR subjects who later on developed schizophrenia revealed a numerically higher accumulation of neuromelanin in the SN compared with those UHR subjects who did not (follow-up length was at least eighteen months). Furthermore, the NM-MRI signal in the ventral SN was significantly and positively correlated with the severity of psychotic symptoms in schizophrenic patients and non-significantly with the severity of attenuated psychotic symptoms in UHR subjects.

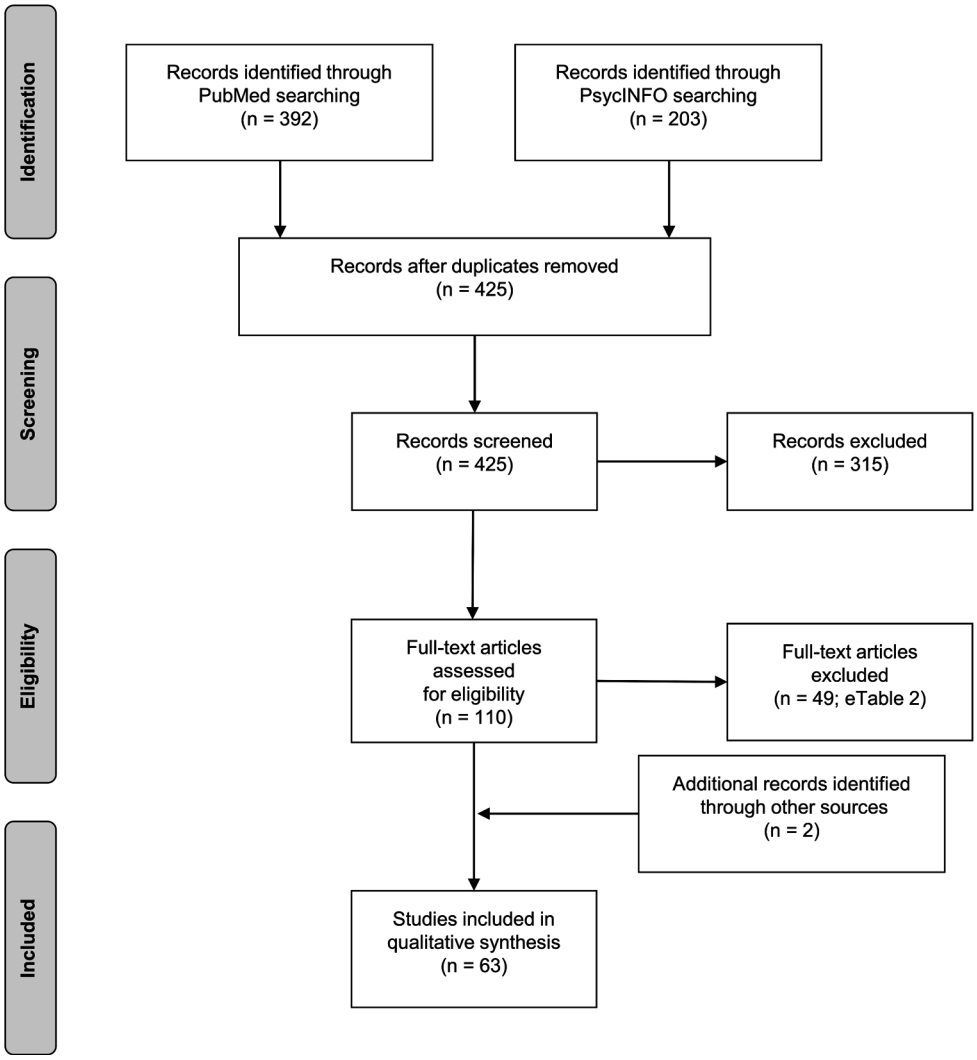

Figure 1. Literature flowchart.

3.1.1.3. Psychosocial stress-induced DA release

Multiple researchers have investigated the effect of psychosocial stress on the dopaminergic system of UHR individuals by exposing subjects to a laboratory psychosocial stress task: the Montreal Imaging Stress Task (MIST).[38] During the MIST, participants need to perform a mental arithmetic task under time pressure and simultaneously they receive negative verbal feedback. Psychosocial stress-induced DA release is determined by calculating the difference in DA $D_{2/3}$ receptor binding between the MIST and a control session (i.e., a mental arithmetic task without time constraints or negative feedback). Schifani et al. (2018, 2019)[39,40] assessed stress-induced DA release

by [11C]FLB457 PET in the dorsolateral prefrontal cortex (dlPFC) and medial prefrontal cortex (mPFC) of UHR (with and without cannabis use), schizophrenic patients, and HC. No differences were found in psychosocial stress-induced DA responses in the dlPFC and mPFC between UHR (without cannabis use), schizophrenic patients, and HC.[40] However, cannabis-using UHR subjects exhibited a lower psychosocial stress-induced DA release in the mPFC than non-using UHR subjects, but not when compared with HC.[39] Furthermore, no differences in psychosocial stress-induced change in binding potential (BP_{ND}) were found among UHR (without cannabis use), cannabis-using UHR, and HC in the dlPFC. Age of first regular cannabis use and the length of cannabis use were, respectively, positively and inversely associated with stress-induced DA release in the mPFC (after adjusting for current cannabis use).[39] As opposed to the findings in the prefrontal cortex (PFC), Mizrahi et al. (2012)[41] found a larger psychosocial stress-induced DA release (assessed with [11C]-(+)-PHNO PET) in the WS and AST of UHR and schizophrenic patients than HC, with no significant differences between UHR and schizophrenic subjects. In addition, cannabis-using UHR subjects showed less DA release in the WS and all its functional subdivisions compared with UHR subjects.[42] The radioligand displacement (i.e., reflecting DA release) in any of the brain regions was not associated with the age at first cannabis use, cannabis lifetime use, or years of cannabis use.[42] Tseng et al. (2018)[43] reported a significant effect of clinical group on psychosocial stress-induced [11C]-(+)-PHNO displacement in the WS, AST, SMST, and the SN, with greater displacement in schizophrenic patients compared with HC. This effect was not observed in the LST. Moreover, no significant differences in nigral [11C]-(+)-PHNO displacement were reported between schizophrenic and UHR subjects or between UHR subjects and HC. Additionally, a greater radiotracer displacement in the SN was found in non-cannabis users relative to cannabis users across all groups. This effect did not differ between diagnostic groups (UHR, schizophrenic patients, or HC). Last of all, in UHR individuals, stress-induced nigral [11C]-(+)-PHNO displacement was negatively associated with the Scale of Prodromal Symptoms (SOPS) negative symptom scores (after adjusting for cannabis use). Overall, psychosocial stress-induced DA release seems not to be altered in extrastriatal regions and increased in striatal regions of UHR compared with HC. The latter is in line with the increased sDSC in UHR, indicating increased presynaptic dopaminergic activity. Furthermore, psychosocial stress-induced DA release in cannabis-using subjects appears not to be the same as in non-using subjects.

3.1.1.4. Cognitive task-induced DA release

One [11C]FLB457 PET study investigated the effects of a cognitive challenge on cortical DA release in UHR and HC individuals.[44] The Wisconsin Card Sorting Test (WCST)

did not significantly result in dissimilarities concerning DA release in the dlPFC or anterior cingulate cortex (ACC) of UHR and HC.

3.1.1.5. Pharmacologically-induced DA release

In previous papers, [^{11}C]-(+)-PHNO has been used to examine stress-induced DA release.[41] Recently, [^{11}C]-(+)-PHNO imaging has been combined with a methylphenidate challenge to examine extrastriatal and striatal intrasynaptic DA release in UHR and HC.[45] A greater methylphenidate-induced ΔBP_{ND} (i.e., DA release induced by methylphenidate) was found in the ventral striatum (VST) of UHR relative to HC. This indicates an excess of DA release in the VST of UHR. This excess of DA release was not found in other striatal (i.e., AST and SMST) or extrastriatal (i.e., thalamus, globus pallidus [GP], and a midbrain region containing the SN and ventral tegmental area [VTA]) brain regions.[45] Two out of fourteen UHR subjects developed schizophrenia within the follow-up period of two years. No significant differences in striatal DA functioning were found between transitioned UHR and non-transitioned UHR subjects.[45] Another pharmacological challenge that has been used to examine changes in synaptic DA concentrations in UHR and HC subjects is the well-validated alpha-methyl-*para*-tyrosine (AMPT) challenge paradigm.[46] Administration of AMPT induces a fast and reversible depletion of DA.[47] In both groups, the administration of AMPT significantly increased the [^{123}I]IBZM BP_{ND} in the striatum, due to DA depletion. However, this increase was not significantly different between UHR and HC. This indicates that the synaptic DA concentration is not altered in UHR individuals. Nevertheless, within the UHR group, the AMPT-induced change in striatal [^{123}I]IBZM BP_{ND} was positively associated with baseline (i.e., pre-AMPT) PANSS total, baseline PANSS positive subscale, and baseline total CAARMS positive subscale scores. No such associations were evident in HC. Furthermore, AMPT significantly decreased PANSS positive subscale scores in UHR subjects. Corresponding to this finding, higher synaptic DA concentrations were predictive of good response of positive symptoms to DA depletion by AMPT (i.e., larger reduction of the PANSS positive subscale score following depletion). In sum, in most brain regions pharmacologically-induced DA release or synaptic DA levels do not differ between UHR and HC.

3.1.1.6. DA $D_{2/3}$ receptor availability

DA $D_{2/3}$ receptor binding in UHR individuals has been studied with regard to multiple brain regions, namely the PFC, striatum, and SN. First of all, a PET study by Schifani et al. (2018)[40] reported a significant effect of group (UHR, schizophrenic patients, and HC) on baseline [^{11}C]FLB457 BP_{ND}, with lower DA $D_{2/3}$ receptor availability in the dlPFC and mPFC of schizophrenic patients compared with UHR subjects. Complementary to these findings, DA $D_{2/3}$ receptor availabilities were compared with

an additional cannabis-using UHR group in a subsequent article.[39] This cannabis-using UHR group consisted of UHR subjects who had a history of cannabis use of at least three times a week for a minimum of two months or they met the Diagnostic and Statistical Manual of Mental Disorders version 5 (DSM-5) criteria for cannabis use disorder. No group differences in baseline [^{11}C]FLB457 BP_{ND} were reported in either the dlPFC or mPFC between UHR, cannabis-using UHR, and HC.[39] Contrary to the results of Schifani and co-workers, Tagore et al. (2019)[44] reported an increased baseline [^{11}C]FLB457 BP_{ND} in UHR compared with HC in the dlPFC, ACC, and mPFC but no differences in the orbitofrontal cortex (OFC). Furthermore, Bloemen et al. (2013)[46] found no differences between UHR and HC in striatal DA $D_{2/3}$ receptor availability as assessed by [^{123}I]IBZM SPECT. By the use of a different PET radiotracer, namely [^{11}C]-(+)-PHNO, Mizrahi et al. (2014)[42] compared the DA $D_{2/3}$ receptor availability between UHR (with no cannabis use) and cannabis-using UHR individuals. They found no difference in [^{11}C]-(+)-PHNO BP_{ND} between UHR and cannabis-using UHR subjects in the WS, any of its functional subdivisions, GP, or SN. Subsequently, Girgis et al. (2019)[45] found no significant differences in [^{11}C]-(+)-PHNO BP_{ND} between UHR and HC in the AST, SMST, VST, thalamus, GP, or a midbrain region containing the SN and VTA. Lastly, Tseng et al. (2018)[43] combined the study samples of Mizrahi (2012, 2013, 2014)[41,42,48] and Suridjan et al. (2013).[49] They reported no differences in DA $D_{2/3}$ receptor availability in the SN between UHR, schizophrenic patients, and HC. The study quality of Tagore et al. (2019),[44] as assessed by the OSQE, did not seem to deviate from the study qualities of the other studies addressing $D_{2/3}$ receptor availabilities in UHR individuals (see eTable 3). Therefore, all articles, except for Tagore et al. (2019),[44] indicated no significant alterations in baseline striatal or extrastriatal DA $D_{2/3}$ receptor availabilities in UHR subjects (regardless of cannabis use) relative to HC.

3.1.2. SPD

Four studies reported on DA imaging data in individuals with SPD.

3.1.2.1. sDSC

A subset (six out of thirty) of the UHR subjects included in the article of Howes et al. (2011)[8] met DSM-4 criteria for SPD. In an exploratory analysis, striatal [^{18}F]F-DOPA uptake in the SPD group was compared with UHR individuals who transitioned to a first-episode psychosis and those who had not developed a frank psychosis. No significant sDSC differences were found between the SPD and UHR transition groups in the WS, AST, SMST, or LST. However, [^{18}F]F-DOPA uptake was elevated in the SPD group relative to HC in the WS, AST, and SMST, and relative to non-transitioned UHR subjects in the AST.

3.1.2.2. Pharmacologically-induced DA release

Amphetamine-induced DA release was larger in the striatum of SPD patients relative to HC.[50] In SPD patients, this release was not correlated with pre-amphetamine positive PANSS scores.[50] Thompson et al. (2020),[51] however, could not confirm these findings. They did not find any differences with regard to amphetamine-induced DA release in the WS, pre-commissural dorsal caudate (pre-DCA), pre-commissural dorsal putamen (pre-DPU), post-commissural caudate (post-CA), post-commissural putamen (post-PU), or VST between SPD patients and HC. Additionally, among SPD patients, scores on the Schizotypal Personality Questionnaire (SPQ) were not significantly correlated with amphetamine-induced ΔBP_{ND} (i.e., DA release induced by amphetamine) in the WS or any of its functional subdivisions.[51] As the mean OSQE scores of both articles were similar (see eTable 3), we can sum up that the results concerning striatal amphetamine-induced DA release in SPD are inconclusive.

3.1.2.3. DA $D_{2/3}$ receptor availability

An [^{123}I]IBZM SPECT study reported no differences in striatal DA $D_{2/3}$ receptor availability between individuals with a diagnosis of SPD and HC.[50] Furthermore, positive PANSS scores did not correlate with DA $D_{2/3}$ receptor availability in SPD patients.[50] In addition, a recently published PET study did also not observe any differences in [^{11}C]raclopride binding in the WS, pre-DCA, pre-DPU, post-CA, post-PU, or VST between SPD patients and HC.[51] Among SPD patients, scores on the SPQ were not significantly correlated with DA $D_{2/3}$ receptor availability in the WS or any of its functional subdivisions.[51] In conclusion, baseline striatal DA $D_{2/3}$ receptor availability seems not to be altered in SPD individuals relative to HC.

3.1.2.4. DA D_1-like receptor availability

Thompson et al. (2014)[52] found no significant group differences in DA D_1-like receptor availability between SPD and HC in the dlPFC, mPFC, and OFC, as measured with [^{11}C]NNC112 PET. Moreover, no significant group differences in DA D_1-like receptor availability between SPD and HC were reported in the WS, pre-DCA, pre-DPU, post-CA, and post-PU.[52] Their results did suggest a higher DA D_1-like receptor availability in the VST of SPD relative to HC, but this group difference did not survive correction for multiple comparisons. In sum, prefrontal and striatal DA D_1-like receptor availability seems not to be altered in SPD individuals relative to HC.

Table 1. Main characteristics and summary of findings in clinical, genetic, and environmental high-risk individuals.

Source	Participants	Males(M)/ Females(F)	Aspect of the DA system	Radiotracer	Main Findings
Allen et al. (2012)[33,a]	UHR (n=20, 3 UHR-TR and 17 UHR-NTR); HC (n=14)	UHR: 10M/10F; HC: 9M/5F	sDSC	[18F]F-DOPA	(1) = sDSC in AST and LST of UHR vs HC; (2) ↑ sDSC in AST of UHR-TR vs UHR-NTR; (3) = sDSC in LST of UHR-TR vs UHR-NTR
Egerton et al. (2013)[34]	UHR (n=26); HC (n=20)	UHR: 14M/12F; HC: 11M/9F	sDSC	[18F]F-DOPA	(1) ↑ sDSC in WS and AST of UHR vs HC; (2) = sDSC in SMST and LST of UHR vs HC[b]; (3) Within UHR, no association between CAARMS or PANSS total scores and sDSC in WS, AST, and SMST
Fusar-Poli et al. (2010)[35,c]	UHR (n=20); HC (n=14)	UHR: 11M/9F; HC: 10M/4F	sDSC	[18F]F-DOPA	(1) ↑ sDSC in AST of UHR vs HC; (2) = sDSC in SMST and LST of UHR vs HC
Howes et al. (2009)[21]	UHR (n=24); SCZ (n=7); HC (n=12)	UHR: 14M/10F; SCZ: 5M/2F; HC: 8M/4F	sDSC	[18F]F-DOPA	(1) ↑ sDSC in WS and AST of UHR vs HC; (2) = sDSC in SMST and LST of UHR vs HC; (3) = sDSC in WS, AST, SMST, and LST of UHR vs SCZ; (4) Positive correlations between sDSC in WS, AST, and SMST and the severity of prodromal symptoms as indexed by total CAARMS and PANSS scores
Howes et al. (2020)[36]	UHR (n=51, 20 UHR-TR and 15 UHR-NTR); HC (n=19)	UHR: 29M/22F; HC: 9M/10F	sDSC	[18F]F-DOPA	(1) = sDSC in WS, AST, SMST, and LST of UHR vs HC (after adjusting for age, gender, and ethnicity); (2) = sDSC of UHR-TR vs UHR-NTR; (3) sDSC in WS and AST predicted the deterioration of psychotic symptoms (after adjusting for age, gender, and ethnicity)
Bloemen et al. (2013)[46]	UHR (n=14); HC (n=15)	UHR: 12M/4F; HC: 13M/2F	(1) DA $D_{2/3}$ receptor availability; (2) AMPT-induced DA release	[123I]IBZM	(1) = BP_{ND} in striatum of UHR vs HC; (2) = AMPT-induced ΔBP_{ND} in striatum of UHR vs HC; Within UHR: (3) $\%\Delta BP_{ND}$ in striatum was positively correlated with baseline total PANSS, CAARMS positive subscale, and PANSS positive subscale scores. No such associations were evident in HC; (4) AMPT significantly decreased PANSS positive subscale scores; (5) High synaptic DA concentrations predicted good response of positive symptoms to DA depletion

Table 1. (Continued).

Source	Participants	Males(M)/ Females(F)	Aspect of the DA system	Radiotracer	Main Findings
Howes et al. (2011)[8,d]	UHR (n=30, 9 UHR-TR, 15 UHR-NTR and 6 UHR-SPD); HC (n=29)	UHR: 17M/13F; HC: 20M/9F	sDSC	[^{18}F]F-DOPA	(1) ↑ sDSC in WS and AST of UHR-TR vs HC and UHR-TR vs UHR-NTR; (2) = sDSC in SMST and LST of UHR-TR vs HC and UHR-TR vs UHR-NTR; (3) = sDSC in WS, AST, SMST, and LST of UHR-NTR vs HC; (4) Within UHR-TR, positive associations between sDSC in WS and total CAARMS and PANSS scores. No such correlations were evident in UHR-NTR; (5) = sDSC in WS, AST, SMST, and LST of UHR-SPD vs UHR-TR; (6) ↑ sDSC in WS, AST, and SMST of UHR-SPD vs HC; (7) = sDSC in LST of UHR-SPD vs HC; (8) ↑ sDSC in AST of UHR-SPD vs UHR-NTR; (9) = sDSC in WS, SMST, and LST of UHR-SPD vs UHR-NTR
Girgis et al. (2019)[45]	UHR (n=14); HC (n=14)	UHR: 9M/5F; HC: 8M/6F	(1) DA $D_{2/3}$ receptor availability; (2) Methylphenidate -induced DA release	[^{11}C]-(+)-PHNO	(1) = BP_{ND} in AST, SMST, VST, SN/VTA, thalamus, and GP of UHR vs HC; (2) ↑ Methylphenidate-induced ΔBP_{ND} in VST of UHR vs HC; (3) = Methylphenidate-induced ΔBP_{ND} in AST, SMST, SN/VTA, thalamus, and GP of UHR vs HC
Schifani et al. (2019)[39]	UHR (n=14); UHR-CU (n=8); HC (n=11)[e]	UHR: 6M/8F; UHR-CU: 7M/1F; HC: 6M/5F	(1) DA $D_{2/3}$ receptor availability; (2) Psychosocial stress-induced DA release	[^{11}C]FLB457	(1) = BP_{ND} in dlPFC and mPFC of UHR vs UHR-CU vs HC; (2) ↓ Psychosocial stress-induced ΔBP_{ND} in mPFC of UHR-CU vs UHR; (3) = Psychosocial stress-induced ΔBP_{ND} in mPFC of UHR-CU vs HC; (4) = Psychosocial stress-induced ΔBP_{ND} in dlPFC of UHR vs UHR-CU vs HC; (5) Length of cannabis use was inversely associated with ΔBP_{ND} in mPFC (after adjusting for current cannabis use); (6) Age of first regular use was positively associated with ΔBP_{ND} in mPFC (after adjusting for current cannabis use)
Kasanova et al. (2018)[61]	FDR (n=16); HC (n=16)	N/A	Cognitive task - induced DA release	[^{18}F]fallypride	= Reward-induced DA release in the L and R i) CNC, ii) putamen, and iii) VST of FDR vs HC

Table 1. (Continued).

Source	Participants	Males(M)/ Females(F)	Aspect of the DA system	Radiotracer	Main Findings
Tagore et al. (2019)[44]	UHR (n=13); HC (n=15)	UHR: 9M/4F; HC: 8M/7F	(1) DA $D_{2/3}$ receptor availability; (2) Cognitive task-induced DA release	[^{11}C]FLB457	(1) ↑ BP_{ND} in control and WCST sessions in dlPFC, ACC, and mPFC of UHR vs HC; (2) = BP_{ND} in control and WCST sessions in OFC of UHR vs HC; (3) = Cognitive task-induced ΔBP_{ND} in dlPFC and ACC of UHR vs HC
Schifani et al. (2018)[40]	UHR (n=14); SCZ (n=14, AP-free); HC (n=12)	UHR: 6M/8F; SCZ: 8M/6F; HC: 7M/5F	(1) DA $D_{2/3}$ receptor availability; (2) Psychosocial stress-induced DA release	[^{11}C]FLB457	(1) ↓ BP_{ND} in dlPFC and mPFC of SCZ vs UHR; (2) = Psychosocial stress-induced ΔBP_{ND} in dlPFC and mPFC of UHR vs SCZ vs HC
Mizrahi et al. (2012)[41]	UHR (n=12); AP-naïve SCZ (n=10); HC (n=12)	UHR: 7M/5F; SCZ: 7M/3F; HC: 7M/5F	Psychosocial stress-induced DA release	[^{11}C]-(+)-PHNO	(1) ↑ Psychosocial stress-induced ΔBP_{ND} in WS and AST in UHR vs HC and SCZ vs HC; (2) = Psychosocial stress-induced ΔBP_{ND} in SMST and LST in UHR vs HC and SCZ vs HC; (3) = Psychosocial stress-induced ΔBP_{ND} in WS, AST, SMST, and LST in UHR vs SCZ; (4) Across all groups, stress-induced [^{11}C]-(+)-PHNO displacement in the striatum was not related to early maternal care
Tseng et al. (2018)[43,f]	UHR (n=24; 12 current cannabis users and 12 nonusers); AP-naïve SCZ (n=9; 4 current cannabis users and 5 nonusers); HC (n=25; 13 current cannabis users and 12 nonusers)	UHR: 13M/11F; SCZ: 6M/3F; HC: 13M/12F	(1) DA $D_{2/3}$ receptor availability; (2) Psychosocial stress-induced DA release	[^{11}C]-(+)-PHNO	(1) = Control session BP_{ND} in SN of UHR vs SCZ vs HC; (2) ↑ Psychosocial stress-induced ΔBP_{ND} in WS, AST, SMST, and SN of SCZ vs HC; (3) = Psychosocial stress-induced ΔBP_{ND} in LST of SCZ vs HC; (4) = Psychosocial stress-induced ΔBP_{ND} in SN of UHR vs SCZ and UHR vs HC; (5) ↑ Psychosocial stress-induced ΔBP_{ND} in SN of non-cannabis users (n=29) vs cannabis-users (n=29) across groups. This effect of cannabis use on psychosocial stress-induced ΔBP_{ND} did not differ between diagnostic groups (UHR, SCZ, and HC); (6) In UHR, psychosocial stress-induced ΔBP_{ND} in SN was negatively associated with SOPS negative symptoms score (after adjusting for cannabis use)

Table 1. (Continued).

Source	Participants	Males(M)/ Females(F)	Aspect of the DA system	Radiotracer	Main Findings
Mizrahi et al. (2014)[42]	UHR (n=12)[g]; UHR-CU (n=12)	UHR: 7M/5F; UHR-CU: 6M/6F	(1) DA $D_{2/3}$ receptor availability; (2) Psychosocial stress-induced DA release	[^{11}C]-(+)-PHNO	(1) = Control task BP_{ND} in WS, AST, LST, SMST, GP, and SN of UHR-CU vs UHR; (2) ↑ Psychosocial stress-induced ΔBP_{ND} in WS, AST, SMST, and LST in UHR-CU vs UHR; (3) No association between psychosocial stress-induced ΔBP_{ND} for any brain region and cannabis lifetime use or years
Thompson et al. (2020)[51]	SPD (n=16); HC (n=16)	SPD: 11M/5F; HC: 12M/4F	(1) DA $D_{2/3}$ receptor availability; (2) Amphetamine-induced DA release	[^{11}C]raclopride	(1) = Baseline BP_{ND} in the WS, pre-DCA, pre-DPU, post-CA, post-PU, and VST of SPD vs HC (adjusted for age); (2) = Amphetamine-induced ΔBP_{ND} in the WS, pre-DCA, pre-DPU, post-CA, post-PU, and VST of SPD vs HC (adjusted for age); (3) In SPD, no significant associations were found between SPQ scores and baseline BP_{ND} or amphetamine-induced ΔBP_{ND} in the WS or any of the striatal subregions (adjusted for age)
Thompson et al. (2014)[52]	SPD (n=18); HC (n=21)	SPD: 12M/6F; HC: 14M/7F	DA D_1-like receptor availability	[^{11}C]NCC112	= DA D_1-like receptor availability in dlPFC, mPFC, OFC, VST, pre-DCA, pre-DPU, post-CU, post-PU, and WS of SPD vs HC
Cassidy et al. (2019)[37]	UHR (n=25); SCZ (n=33, unmedicated); HC1 (n=15 matched to UHR); HC2 (n=30, matched to SCZ)	UHR: 13M/12F; SCZ: 23M/10F; HC1: 9M/6F; HC2: 18M/12F	Neuromelanin	N/A	(1) = NM in SN of UHR vs HC or UHR vs SCZ; (2) ↑ NM in SN of UHR-TR vs UHR-NTR; (3) NM in SN was positively and significantly correlated with the severity of psychotic symptoms in SCZ and non-significantly with the severity of attenuated psychotic symptoms in UHR subjects
Abi-Dargham et al. (2004)[50]	SPD (n=13); HC (n=13)	SPD: 10M/3F; HC: 9M/4F	(1) DA $D_{2/3}$ receptor availability; (2) Amphetamine-induced DA release	[^{123}I]IBZM	(1) = Baseline DA $D_{2/3}$ receptor availability in the striatum of SPD vs HC; (2) ↑ Amphetamine-induced DA release in the striatum of SPD vs HC; (3) In SPD, no correlations were found between baseline positive symptoms and DA $D_{2/3}$ receptor availability or amphetamine-induced DA release

Table 1. (Continued).

Source	Participants	Males(M)/ Females(F)	Aspect of the DA system	Radiotracer	Main Findings
Soliman et al. (2008)[53]	Positive schizotypes (n=9); Negative schizotypes (n=7); HC (n=10)	Positive schizotypes: 2M/7F; Negative schizotypes: 1M/6F; HC: 1M/9F	(1) DA $D_{2/3}$ receptor availability; (2) Psychosocial stress-induced DA release	[^{11}C]raclopride	(1) = Non-stress BP_{ND} in the striatum of positive schizotypes vs negative schizotypes vs HC; (2) Psychosocial stress induced a decrease in BP_{ND} in the bilateral VST, CNC, and putamen of negative schizotypes. This was not evident in positive schizotypes or HC; (3) = Psychosocial stress-induced ΔBP_{ND} in the striatum of positive schizotypes vs negative schizotypes vs HC; (4) Subjects with low maternal care scores showed the highest stress-induced striatal DA release across all participants
Chen et al. (2012)[58]	Healthy volunteers (N=55)	34M/21F	DA $D_{2/3}$ receptor availability	[^{123}I]IBZM	(1) No correlation between total SPQ scores and striatal DA $D_{2/3}$ receptor availability; (2) Positive correlation between disorganized factor of the SPQ and R striatal DA $D_{2/3}$ receptor availability
Woodward et al. (2011)[57]	Healthy individuals (N=63)	32M/31F	Amphetamine-induced DA release	[^{18}F]fallypride	(1) Amphetamine-induced DA release in the WS and AST was positively correlated with overall schizotypal traits. This was not evident for extrastriatal regions (i.e., amygdala, hippocampus, and thalamus); (2) Voxel-wise analysis identified correlations between overall schizotypal traits and DA release in the L and R striatum, L middle frontal gyrus, and L supramarginal gyrus (corrected for age, gender, and cohort)
Brunelin et al. (2010)[64]	FDR (n=8); HC (n=10)	FDR: 5M/3F; HC: 6M/4F	Metabolic stress-induced DA release	[^{11}C]raclopride	(1) = Pre-2DG BP_{ND} in the WS of FDR vs HC; (2) In HC, BP_{ND} pre-2DG > BP_{ND} post-2DG in the striatum. In FDR, BP_{ND} pre-2DG = BP_{ND} post-2DG in the striatum. In HC and FDR, BP_{ND} pre-2DG = BP_{ND} post-2DG in the Nacc; (3) In FDR, ΔBP_{ND} L > ΔBP_{ND} R in the striatum and Nacc; (4) In both groups, the level of Nacc asymmetry after stress induction was associated with positive schizotypy scores
Boot et al. (2010)[70]	22q11DS (n=12); HC (n=12)	22q11DS: 5M/7F; HC: 5M/7F	DA $D_{2/3}$ receptor availability	[^{123}I]IBZM	= Striatal BP_{ND} of 22q11DS vs HC

Table 1. (Continued).

Source	Participants	Males(M)/ Females(F)	Aspect of the DA system	Radiotracer	Main Findings
Huttunen et al. (2008)[22]	FDR (n=17, 7 children of SCZ and 10 siblings of SCZ); HC (n=17)	FDR: 3M/14F; HC: 6M/11F	sDSC	[18F]F-DOPA	(1) ↑ sDSC in the L and R CNC and R putamen of FDR vs HC; (2) ↑ sDSC in the R vs L CNC across both groups; (3) = sDSC in CNC and putamen of FDR-children vs FDR-siblings; (4) Within FDR, no correlation between PANSS scores and sDSC
Lataster et al. (2014)[62]	FDR (n=14, 7 siblings, 6 parents, and 1 child of a psychotic patient); HC (n=10)	FDR: 7M/7F; HC: 8M/2F	(1) DA $D_{2/3}$ receptor availability; (2) Psychosocial stress-induced DA release	[18F]fallypride	(1) = BP_{ND} in the L and R vmPFC of FDR vs HC; (2) = Psychosocial stress-induced DA release in L and R vmPFC of FDR vs HC
Lee et al. (2008)[63]	Unaffected relatives (n=11, 9 with two or more first- or second-degree relatives with SCZ and 2 MZ co-twins); HC (n=11)	Unaffected relatives: 3M/8F; HC: 3M/8F	DA $D_{2/3}$ receptor availability	[11C]raclopride	(1) = Binding in the L and R i) putamen and ii) CNC of unaffected relatives vs HC; (2) No correlation between genetic load and [11C]raclopride binding; (3) ↑ Binding in R vs L putamen of HC. This was not the case in unaffected relatives
Shotbolt et al. (2011)[60]	Twin pairs discordant for SCZ (n=7, 1 MZ and 6 DZ); HC twin pairs (n=10, all DZ)	Twin pairs discordant for SCZ: 5M/2F; HC twin pairs: 5M/5F	sDSC	[18F]F-DOPA	= sDSC in the WS, AST, LST, and SMST of twins with SCZ vs non-psychotic co-twins vs HC twins
Eisenstein et al. (2017)[68]	Siblings of individuals with SCZ (n=10); SCZ (n=3); HC (n=10)	SIB: 4M/6F; SCZ: 2M/1F; HC: 6M/4F	DA $D_{2/3}$ receptor availability	[11C]NMB	= BP_{ND} in dorsal and ventral striatum of SIB vs SCZ vs HC

Table 1. (Continued).

Source	Participants	Males(M)/ Females(F)	Aspect of the DA system	Radiotracer	Main Findings
Hirvonen et al. (2005)[65]	Healthy co-twins from pairs discordant for SCZ (n=11, 6 MZ and 5 DZ); HC twins (n=14, 7 pairs: 4 MZ and 3 DZ)	MZ co-twins: 4M/2F; DZ co-twins: 3M/2F; HC twins: 8M/6F	DA $D_{2/3}$ receptor availability	[^{11}C]raclopride	(1) ↑ Binding in the CNC of MZ co-twins vs HC twins and DZ co-twins; (2) = Binding in the CNC of DZ co-twins vs HC twins; (3) = Binding in the putamen and thalamus of MZ co-twins vs DZ co-twins vs HC twins
Kuepper et al. (2013)[66]	FDR (n=8); Patients with a psychotic disorder (n=8); HC-CU (n=9)[h]	FDR: 5M/3F; Patients: 6M/2F; HC-CU: 5M/4F	THC-induced DA release	[^{18}F]fallypride	(1) In FDR and patients, pre-THC BP_{ND} > post-THC BP_{ND} in the striatum. This was not the case for HC-CU; (2) ↑ THC-induced DA release in L CNC of FDR and patients vs HC (corrected for gender, age, nicotine use, alcohol use, use of other drugs and other medication, and frequency of cannabis use). No difference between FDR and patients in this region
Hirvonen et al. (2006)[67,i]	SCZ (n=9); MZ co-twins (n=6); DZ co-twins (n=5); HC twins (n=13, 7 pairs: 4 MZ and 3 DZ)	SCZ: 5M/4F; MZ co-twins: 4M/2F; DZ co-twins: 3M/2F; HC twins: 8M/5F	DA D_1-like receptor availability	[^{11}C]SCH 23390	(1) ↑ Binding in the mPFC, STG, and AG of MZ co-twins vs HC twins; (2) ↓ Binding in the CNC, putamen, dlPFC, mPFC, OFC, ACC, AG, SMG, STG, MTG, and insular cortex of SCZ vs MZ co-twins and DZ co-twins
Hirvonen et al. (2006)[69,j]	Healthy co-twins from pairs discordant for SCZ (n=11, 6 MZ and 5 DZ); HC twins (n=13, 7 pairs: 4 MZ and 3 DZ)	MZ co-twins: 4M/2F; DZ co-twins: 3M/2F; HC twins: 8M/5F	DA D_1-like and DA $D_{2/3}$ receptor availability	(1) [^{11}C]SCH 23390; (2) [^{11}C]raclopride	= DA D_1-like/$D_{2/3}$ ratio in the putamen and CNC of MZ co-twins vs DZ co-twins vs HC twins

Table 1. (Continued).

Source	Participants	Males(M)/ Females(F)	Aspect of the DA system	Radiotracer	Main Findings
Sevy et al. (2008)[79]	CD in early full remission (n=6); HC (n=6)	CD: 6M/0F; HC: 6M/0F	DA $D_{2/3}$ receptor availability	[^{11}C]raclopride	= Binding in L and R i) CNC and ii) putamen of CD vs HC
Dahoun et al. (2018)[71,k]	Serine homozygotes (AA; n=46); Cysteine homozygotes (TT; n=11); Cysteine Heterozygotes (AT: n=45)	AA: 21M/25F; TT/AT: 35M/21F	sDSC	[^{18}F]F-DOPA	↑ sDSC in AA vs TT/AT
Albrecht et al. (2013)[72]	CU (n=10); HC (n=8)	CU: 10M/0F; HC: 8M/0F	DA $D_{2/3}$ receptor availability	[^{11}C]raclopride	(1) = Striatal BP_{ND} of CU vs HC; (2) Within CU, BP_{ND} is negatively associated with recent cannabis use per day
Urban et al. (2012)[75]	CD (n=16); HC (n=16)	CD: 15M/1F; HC: 14M/2F	(1) DA $D_{2/3}$ receptor availability; (2) Amphetamine-induced DA release	[^{11}C]raclopride	(1) = BP_{ND} in the WS, VST, AST (pre-DCA, pre-DPU, and post-CA), and SMST (post-PU) of CD vs HC; (2) = Amphetamine-induced ΔBP_{ND} in the WS, VST, AST, and SMST of CD vs HC; (3) No correlations between baseline BP_{ND} or amphetamine-induced ΔBP_{ND} and i) the severity of cannabis use and ii) duration of abstinence; (4) In CD, ↓ amphetamine-induced ΔBP_{ND} in the AST and pre-DCA correlated with earlier age of onset of cannabis use (when adjusting for current age)
Rogdaki et al. (2021)[23]	22q11DS (n=21); 22q11DUP (n=12); HC (n=26)	22q11DS: 7M/14F; 22q11DUP: 7M/5F; HC: 11M/15F	sDSC	[^{18}F]F-DOPA	(1) ↑ sDSC in WS, AST, SMST, and LST of 22q11DS vs HC and 22q11DUP (adjusted for age and injected activity); (2) Across 22q11DS and 22q11DUP, there was a significant positive relationship between striatal [^{18}F]F-DOPA uptake and CAARMS positive symptom severity ratings. This was not the case for the severity of anxiety and depressive symptoms

Table 1. (Continued).

Source	Participants	Males(M)/ Females(F)	Aspect of the DA system	Radiotracer	Main Findings
Dahoun et al. (2019)[73]	Healthy individuals (N=41; Serine homozygotes [AA; n=19], Cysteine homozygotes and heterozygotes [TT/AT; n=22]; Leucine homozygotes [CC; n=31], Phenylalanine homozygotes and heterozygotes [TC/TT; n=10]; Arginine homozygotes [GG; n=21], Glutamine homozygotes and heterozygotes [AG/AA; n=20])	AA: 9M/10F; TT/AT: 16M/6F; CC: 18M/13F; TC/TT: 7M/3F; GG: 14M/7F; AG/AA: 9M/11F	DA $D_{2/3}$ receptor availability	[^{11}C]-(+)-PHNO	= BP_{ND} in striatum, CNC, and putamen of AA vs TT/AT, CC vs TC/TT, and GG vs AG/AA
van Duin et al. (2018)[74]	22q11DS (n=12); HC (n=16)	22q11DS: 4M/8F; HC: 4M/12F	(1) DA $D_{2/3}$ receptor availability; (2) Cognitive task-induced DA release	[^{18}F]fallypride	(1) = BP_{ND} in mean, L, and R i) VST, ii) putamen, and iii) CNC of 22q11DS vs HC (while including IQ as a covariate); (2) = Reward-induced ΔBP_{ND} in mean, L, and R i) VST, ii) putamen, and iii) CNC of 22q11DS vs HC (while including IQ as a covariate)
Vingerhoets et al. (2018)[77,1]	22q11DS (n=15); UHR (n=16); HC (n=11)	22q11DS: 6M/9F; UHR: 12M/4F; HC: 4M/7F	DA $D_{2/3}$ receptor availability	[^{123}I]IBZM	= Mean striatal BP_{ND} of 22q11DS vs UHR vs HC (adjusted for age and gender)

Table 1. (Continued).

Source	Participants	Males(M)/ Females(F)	Aspect of the DA system	Radiotracer	Main Findings
Stokes et al. (2012)[76,m]	CU (n=10); HC (n=10)	CU: 6M/4F; HC: 9M/1F	DA $D_{2/3}$ receptor availability	[^{11}C]raclopride	(1) = BP_{ND} in the WS, AST, SMST, and LST of CU vs HC (adjusted for current nicotine cigarette smoking status and age); (2) No correlations between BP_{ND} in the WS, AST, SMST, or LST and i) frequency of lifetime cannabis use, ii) years of cannabis use, iii) age of first use, and iv) duration since last cannabis use
Bloomfield et al. (2014)[78]	CU (n=19, 5 met DSM-4 criteria for cannabis dependence and 5 met DSM-4 criteria for cannabis abuse); NCU (n=19)	CU: 17M/2F; NCU: 17M/2F	sDSC	[^{18}F]F-DOPA	(1) ↓ sDSC in the WS, AST, and LST of CU vs NCU (after covarying for non-cannabis drug use). Not evident in the SMST; (2) ↓ sDSC in the WS and AST of CU-dependence/abuse vs CU-non-dependence/non-abuse; ↓ sDSC in the WS of CU-dependence/abuse vs NCU. = sDSC in the WS of CU-non-dependence/non-abuse vs NCU; (3) Within CU, lower sDSC in the WS, AST, and SMST was associated with ↑ levels of current cannabis use; (4) Within CU, younger age at first cannabis exposure was associated with ↓ sDSC in the WS and AST (adjusted for current age)
Volkow et al. (2014)[80]	MA (n=24); HC (n=24)	MA: 12M/12F; HC: 12M/12F	(1) DA $D_{2/3}$ receptor availability; (2) Methylphenidate -induced DA release	[^{11}C]raclopride	(1) = BP_{ND} in the striatum (CNC, putamen, and VST) of MA vs HC; (2) $BP_{ND\text{-}MP} < BP_{ND\text{-}placebo}$ in the striatum of MA and HC; (3) = MP-induced DA release in the striatum of MA vs HC; (4) ↑ MP-induced DA release in the midbrain of MA vs HC
Tomasi et al. (2015)[92]	CA/CD (n=18); HC (n=14)	CA/CD: 9M/9F; HC: 9M/5F	DA $D_{2/3}$ receptor availability	[^{11}C]raclopride	= BP_{ND} in the putamen, GP, dorsal CNC, and VST of CA/CD vs HC
van Duin et al. (2020)[91]	22q11DS (n=14)[n]; HC (n=16)	22q11DS: 6M/8F; HC: 4M/12F	DA $D_{2/3}$ receptor availability	[^{18}F]fallypride	(1) ↓ BP_{ND} in the ACG of 22q11DS vs HC. = BP_{ND} in the PFC, OFC, and ACC of 22q11DS vs HC; (2) Within 22q11DS, there were no associations between BP_{ND} in the PFC, OFC, ACC, or ACG and PANSS scores

Table 1. (Continued).

Source	Participants	Males(M)/ Females(F)	Aspect of the DA system	Radiotracer	Main Findings
Bloomfield et al. (2019)[94]	HA (n=17); LA (n=17)	HA: 9M/8F; LA: 8M/9F	sDSC	[^{18}F]F-DOPA	↓ sDSC in the WS, AST, and LST of HA vs LA. After co-varying for current cigarette use, the group difference only remained significant in the LST
van de Giessen et al. (2017)[81]	CD (n=11); HC (n=12)	CD: 7M/4F; HC: 8M/4F	(1) DA $D_{2/3}$ receptor availability; (2) Amphetamine-induced DA release	[^{11}C]-(+)-PHNO	(1) = BP_{ND} in the WS, AST, SMST, LST, GP, thalamus, and midbrain of CD vs HC; (2) ↓ Amphetamine-induced ΔBP_{ND} in the WS, AST, SMST, and GP of CD vs HC; (3) No correlations between amphetamine-induced ΔBP_{ND} and the severity of cannabis use in any striatal subdivision
Mizrahi et al. (2013)[48]	CU (n=13); HC (n=12)	CU: 6M/7F; HC: 7M/5F	(1) DA $D_{2/3}$ receptor availability; (2) Psychosocial stress-induced DA release	[^{11}C]-(+)-PHNO	(1) ↑ BP_{ND} in the WS, AST, LST, and SMST of CU vs HC. After excluding cannabis users from the analysis who reported having used cannabis less than 8 hours before the PET-scan, no differences in BP_{ND} were observed; (2) = BP_{ND} in the GP and SN of CU vs HC; (3) ↓ Psychosocial stress-induced ΔBP_{ND} in the GP of CU vs HC. After excluding cannabis users from the analysis who reported having used cannabis less than 8 hours before the PET-scans, the difference in tracer displacement did not remain significant; (4) = Psychosocial stress-induced ΔBP_{ND} in the WS, AST, LST, SMST, or SN of CU vs HC
Leroy et al. (2012)[82]	CS (n=13); TS (n=14); NS (n=11)	CS: 13M/0F; TS: 14M/0F; NS: 11M/0F	DAT availability	[^{11}C]PE21	(1) ↓ BP_{ND} in the CNC, putamen, VST, midbrain, grey and white matter of the cingulate gyrus, and several thalamic nuclei of CS and TS vs NS. No differences between CS and TS; (2) In CS and TS, no correlations were found between BP_{ND} and i) the age of onset or history use or ii) the number of joints and/or cigarettes smoked per day
Hoexter et al. (2012)[89]	PTSD (n=21); Resilient controls (n=21)	PTSD: 4M/17F; Resilient controls: 4M/17F	DAT availability	[^{99m}Tc]-TRODAT-1	↓ Binding in the L, R, and mean striatum of resilient controls vs PTSD

Table 1. (Continued).

Source	Participants	Males(M)/ Females(F)	Aspect of the DA system	Radiotracer	Main Findings
Stokes et al. (2009)[83]	Healthy individuals with previous experience of cannabis use (N=13)	7M/6F	THC-induced DA release	[^{11}C]raclopride	THC-induced ΔBP_{ND} in the striatum was unrelated to the extent of previous cannabis exposure
Stokes et al. (2010)[84,o]	Healthy individuals with previous experience of cannabis use (N=13)	7M/6F	THC-induced DA release	[^{11}C]raclopride	(1) THC-induced ΔBP_{ND} in the RMFG, LSFG, and LSTG did not correlate with the lifetime frequency of cannabis use
Gevonden et al. (2014)[86]	SHI (n=19); HC (n=19)	SHI: 3M/16F; HC: 3M/16F	(1) DA $D_{2/3}$ receptor availability; (2) Dexampheta-mine-induced DA release	[^{123}I]IBZM	(1) = Striatal BP_{ND} of SIH vs HC; (2) ↑ Dexamphetamine-induced striatal ΔBP_{ND} in SIH vs HC (adjusted for age and tobacco smoking)
Oswald et al. (2014)[87]	Healthy individuals (N=28)	19M/9F	(1) DA $D_{2/3}$ receptor availability; (2) Amphetamine-induced DA release	[^{11}C]raclopride	(1) Pre-amphetamine BP_{ND} > post-amphetamine BP_{ND} in the VST; (2) Positive association between ETI scores and pre-amphetamine BP_{ND} in the i) VST, ii) anterior CNC, and iii) anterior putamen of males; (3) Positive relation between ETI scores and amphetamine-induced ΔBP_{ND} in the VST. This was not evident in the anterior and posterior i) CNC or ii) putamen; (4) The relation between ETI scores and amphetamine-induced ΔBP_{ND} in the VST was mediated by PSS scores
Montgomery et al. (2006)[96]	Healthy volunteers (N=14)	9M/5F	Psychosocial stress-induced DA release	[^{11}C]raclopride	No relation between maternal care and psychosocial stress-induced DA release in the dorsal striatum and VST

Table 1. (Continued).

Source	Participants	Males(M)/ Females(F)	Aspect of the DA system	Radiotracer	Main Findings
Egerton et al. (2017)[85]	$Immigrant_{CA}$ (n=26, 9 $first\text{-}generation_{CA}$, 8 $second\text{-}generation_{CA}$, and 9 data unavailable); $Nonimmigrant_{CA}$ (n=30); $Immigrant_{UK}$ (n=32, 12 $first\text{-}generation_{UK}$ and 20 $second\text{-}generation_{UK}$); $Nonimmigrant_{UK}$ (n=44)	$Immigrant_{CA}$: 14M/12F; $Nonimmigrant_{CA}$: 18M/12F; $Immigrant_{UK}$: 15M/17F; $Nonimmigrant_{UK}$: 29M/15F	(1) sDSC; (2) Psychosocial stress-induced DA release	(1) [^{18}F]F-DOPA; (2) [^{11}C]-(+)-PHNO	(1) ↑ Stress-induced DA release in the WS, AST, and LST of $immigrant_{CA}$ vs $nonimmigrant_{CA}$ (adjusted for cannabis use); (2) ↑ Stress-induced striatal DA release in $first\text{-}generation_{CA}$ vs $nonimmigrant_{CA}$. = Stress-induced striatal DA release in $second\text{-}generation_{CA}$ vs $nonimmigrant_{CA}$ and $first\text{-}generation_{CA}$; (3) ↑ sDSC in the WS and SMST of $immigrant_{UK}$ vs $nonimmigrant_{UK}$ (adjusted for cannabis use); (4) ↑ sDSC in $second\text{-}generation_{UK}$ vs $nonimmigrant_{UK}$. = sDSC in $first\text{-}generation_{UK}$ vs $nonimmigrant_{UK}$ and $second\text{-}generation_{UK}$
Egerton et al. (2016)[24]	UHR (n=47); HC (n=20)	UHR: 27M/20F; HC: 12M/8F	sDSC	[^{18}F]F-DOPA	(1) Across UHR and HC, ↑ sDSC in the WS and AST of subjects who experienced severe physical, sexual abuse, or more than two family arrangements during childhood compared with those who had not (adjusted for age, gender, alcohol drinking or smoking status, and use of cannabis, ecstasy, cocaine, amphetamine, or ketamine); (2) Across UHR and HC, ↑ sDSC in the SMST of subjects who experienced more than two family arrangements compared with those who had not; (3) No significant interaction effect between group (UHR and HC) and childhood adversity on sDSC; (4) = sDSC in the WS, AST, SMST, and LST of UHR vs HC
Pruessner et al. (2004)[38]	High early life maternal care (n=5); Low early life maternal care (n=5)	9M/1F	Psychosocial stress-induced DA release	[^{11}C]raclopride	Significant psychosocial stress-induced DA release in the VST and putamen of subjects with low, but not high, self-reported early life maternal care

Table 1. (Continued).

Source	Participants	Males(M)/ Females(F)	Aspect of the DA system	Radiotracer	Main Findings
Dahoun et al. (2019)[88]	Healthy individuals (N=24)	13M/11F	(1) DA $D_{2/3}$ receptor availability; (2) Dexampheta-mine-induced DA release	[^{11}C]-(+)-PHNO	(1) Pre-dexamphetamine BP_{ND} > post-dexamphetamine BP_{ND} in the VST, AST, and SMST; (2) Pre-dexamphetamine psychotic symptoms < post-dexamphetamine psychotic symptoms; (3) No relation between pre-dexamphetamine BP_{ND} in the VST and childhood trauma; (4) Childhood trauma was negatively associated with ventral striatal DA release; (5) The interaction between childhood trauma and dexamphetamine-induced DA release in the VST predicted dexamphetamine-induced positive psychotic symptoms; (6) No indirect effect of childhood trauma on dexamphetamine-induced positive psychotic symptoms through dexamphetamine-induced DA release
Kasanova et al. (2016)[90]	NAPD (n=12); HC (n=12)	NAPD: 8M/4F; HC: 8M/4F	Psychosocial stress-induced DA release	[^{18}F]fallypride	(1) Within the HC group, the spatial extent of the psychosocial stress-induced DA release in the mPFC was positively associated with early and late childhood trauma scores. This was not the case for the NAPD group; (2) No significant relations were found between childhood trauma scores and psychosocial stress-induced DA release in either of the groups with regard to the vmPFC or dmPFC
Taurisano et al. (2014)[59]	GG (n=17); GT (n=9)	GG: 8M/9F; GT: 5M/4F	DA $D_{2/3}$ receptor availability	[^{123}I]IBZM	In GT, [^{123}I]IBZM binding was positively correlated with SPQ scores in the R putamen. This association was not present in GG
Schalbroeck et al. (2021)[95]	Healthy individuals (N=22)	14M/8F	sDSC	[^{18}F]F-DOPA	Negative association between childhood trauma and sDSC in the WS, AST, and SMST (adjusted for gender, age, scanner type, and smoking status)

Caption: next page.

Table 1. (Pages 42-55).

Abbreviations: ACC, anterior cingulate cortex; ACG, anterior cingulate gyrus; AG, angular gyrus; AMPT, alpha-methyl-*para*-tyrosine; AP, antipsychotic medication; AST, associative striatum; BP_{ND}, non-displaceable binding potential; CAARMS, Comprehensive Assessment of At-Risk Mental State; CA, cannabis abusers; CD, cannabis-dependent subjects; CNC, caudate nucleus; CS, cannabis-dependent smokers; CU, cannabis users; CU-HC, healthy cannabis users; DA, dopamine; DAT, dopamine transporter; dlPFC, dorsolateral prefrontal cortex; dmPFC, dorsomedial prefrontal cortex; DZ, dizygotic twins; ETI, Early Trauma Inventory; FDR, first degree relatives of psychotic patients; GP, globus pallidus; HA, participants with high cumulative exposure to psychosocial adversity; HC, healthy controls; IQ, intelligence quotient; L, left; LA, participants with low cumulative exposure to psychosocial adversity; LSFG, left superior frontal gyrus; LST, limbic striatum; LSTG, left superior temporal gyrus; MA, marijuana abusers; MTG, middle temporal gyrus; MP, methylphenidate; mPFC, medial prefrontal cortex; MZ, monozygotic twins; Nacc, nucleus accumbens; NCU, non-cannabis users; NM, neuromelanin; NS, non-smokers; OFC, orbitofrontal cortex; PANSS, positive and negative syndrome scale; PFC, prefrontal cortex; post-CA, postcommissural caudate; post-PU, postcommissural putamen; pre-DCA, precommissural dorsal caudate; pre-DPU, precommissural dorsal putamen; PSS, Perceived Stress Scale; RL, reinforcement learning; R, right; RMFG, right middle frontal gyrus; SCZ, schizophrenic patients; sDSC, striatal dopamine synthesis capacity; SHI, severe hearing impairment; SIB, sibling of schizophrenic patients; SMG, supramarginal gyrus; SMST, sensorimotor striatum; SN, substantia nigra; SN/VTA, a midbrain region including the SN and ventral tegmental area; SOPS, Scale of Prodromal Symptoms; SPD, schizotypal personality disorder; SPQ, schizotypal personality questionnaire; STG, superior temporal gyrus; THC, delta-9-tetrahydrocannabinol; TS, tobacco-dependent smokers; UHR, ultra-high risk individuals; UHR-CU, cannabis-using UHR individuals; UHR-TR, UHR transition group; UHR-NTR, UHR non-transition group; UHR-SPD, UHR individuals with SPD; WCST, Wisconsin Card Sorting Test; WS, whole striatum; vmPFC, ventromedial prefrontal cortex; VST, ventral striatum; ↑, increase; ↓, decrease; =, no difference; <, smaller compared with; >, larger compared with; 2DG, 2-Deoxy-D-Glucose; 22q11DS, 22q11.2 deletion syndrome; 22q11DUP, 22q11.2 duplication syndrome.

[a]The PET data from fourteen subjects has been previously reported.[21] [b]When the cohorts of Egerton et al. (2013)[34] and Howes et al. (2009)[21] were combined, a significant elevation in sDSC was found in the WS, AST, and SMST of UHR vs HC. However, this was no longer significant after removing individuals with a SPD, individuals on AP, and individuals who reported any recreation drug use. [c]The PET data from eight out of twenty UHR subjects and five out of fourteen HC have been previously reported.[21] [d]Follow-up data of Howes et al. (2009).[21] [e]UHR and HC samples overlap with Schifani et al. (2018).[40] [f]Cohorts of previous studies were combined.[41,42,48,49] [g]UHR sample overlaps with Mizrahi et al. (2012).[41] [h]All subjects used cannabis at least once in the previous twelve months. [i]The study cohort overlaps with Hirvonen et al. (2005).[65] [j]Same study cohort as Hirvonen et al. (2005, 2006).[65,67] [k]Part of the imaging data has been previously reported.[78,122,123] [l]The same UHR cohort as Bloemen et al. (2013)[46] and the same 22q11 cohort as Boot et al. (2010).[70] [m]Data of cannabis users is a combined sample from Stokes et al. (2009, 2010).[83,84] However, these articles focus on striatal DA release and frontal DA $D_{2/3}$ receptor availability, respectively. Furthermore, data of HC is not reported in one of the included articles in this review. [n]Two additional 22q11 individuals were added to the study cohort of van Duin et al. (2018).[74] [o]An existing dataset was used.[83] However, extrastriatal results have not been published before.

3.1.3. Schizotypal traits in healthy individuals

Four studies reported on DA imaging data with a focus on schizotypal personality traits in healthy individuals.

3.1.3.1. Psychosocial stress-induced DA release

Soliman et al. (2008)[53] investigated psychosocial stress-induced DA release in healthy individuals at elevated risk for psychosis by investigating the DA $D_{2/3}$ receptor availability during the MIST and a non-stress condition. Two groups of at-risk individuals were included: individuals with elevated scores on either the negative (negative schizotypes) or positive (positive schizotypes) symptom dimensions of the physical anhedonia and perceptual aberration scales, respectively. Both of these dimensions have been associated with increased rates of psychosis in longitudinal studies.[54-56] A significant psychosocial stress-induced decrease in [^{11}C]raclopride binding was found in the bilateral VST, caudate nucleus (CNC), and putamen in negative schizotypes only. No such psychosocial stress-induced reduction in binding was evident in positive schizotypes or HC. Also, no differences in psychosocial stress-induced ΔBP_{ND} (i.e., DA release induced by psychosocial stress) were found among HC, positive, and negative schizotypes in the striatum.

3.1.3.2. Pharmacologically-induced DA release

In healthy volunteers, amphetamine-induced DA release in the WS and AST was positively correlated with overall schizotypal traits.[57] This was not evident in extrastriatal regions, such as the amygdala, hippocampus, or thalamus. However, a voxel-wise analysis did identify associations between overall schizotypal traits and amphetamine-induced DA release in the left middle frontal gyrus and left supramarginal gyrus, as well as, the left and right striatum (corrected for age and gender). Furthermore, amphetamine-induced DA release was correlated with the disorganized subscore of the SPQ in various cortical and subcortical regions (i.e., amygdala, thalamus, WS, and its functional subdivisions). The disorganized factor of the SPQ predominantly contains subscales that belong to the positive symptoms of schizotypy. Thus, striatal amphetamine-induced DA release appears to be related to schizotypal traits in healthy individuals.

3.1.3.3. DA $D_{2/3}$ receptor availability

The previously mentioned PET study of Soliman et al. (2008)[53] also investigated baseline DA $D_{2/3}$ receptor availability and found no differences in striatal [^{11}C]raclopride binding between HC, positive, and negative schizotypes. Additionally, Chen et al. (2012)[58] examined whether increased levels of striatal DA $D_{2/3}$ receptors were related to elevated levels of schizotypal features in healthy volunteers. No correlation was found between total scores on the SPQ and DA $D_{2/3}$ receptor availability in the striatum.[58] Nevertheless, a positive relation was observed between right striatal DA $D_{2/3}$ receptor availability and the disorganized factor of the SPQ. Furthermore, Taurisano et al. (2014)[59] investigated how a polymorphism in the DA D_2

gene (DRD2 rs1076560) and schizotypy scores influenced striatal DA $D_{2/3}$ signalling in healthy individuals. A significant interaction between SPQ scores and DRD2 genotype was found in the right putamen. Namely, [^{123}I]IBZM binding was positively correlated with SPQ scores in individuals with the DRD2 polymorphism, while no such association was found in individuals without the DRD2 polymorphism. In short, healthy individuals with schizotypal personalities seem not to have altered striatal DA $D_{2/3}$ receptor binding. However, individual differences with regard to different genotypes may exist.

3.2. Genetic high-risk groups

The effects of genetic liability to schizophrenia on various aspects of the dopaminergic system have been investigated in eighteen studies.

3.2.1. Relatives

3.2.1.1. sDSC

Shotbolt et al. (2011)[60] found no differences in sDSC in the WS, AST, LST, and SMST between schizophrenic patients, their non-affected co-twins, and HC twins. In contrast to these findings, Huttunen et al. (2008)[22] reported an elevated sDSC in the left and right CNC and right putamen of first-degree relatives of patients with schizophrenia compared with HC. Furthermore, a significant effect of hemisphere (right > left) was found in the CNC across both groups. Seven out of seventeen relatives were children of a patient with schizophrenia, while the others were siblings of schizophrenic patients. A preliminary analysis revealed no differences in [^{18}F]F-DOPA uptake in the CNC or putamen between these two subgroups (children vs siblings). Within the group of first-degree relatives, PANSS scores did not correlate with sDSC.[22] Although the methodological quality of Huttunen et al. (2008)[22] was slightly lower than that of Shotbolt et al. (2011)[60] (total OSQE score of six and eight, respectively; see eTable 3), we cannot conclude that sDSC group differences between relatives of psychotic patients and HC are uniform.

3.2.1.2. Psychosocial stress-induced DA release

Psychosocial stress, induced by the MIST, did not result in differences regarding DA release in the left or right vmPFC of first-degree relatives of patients with a psychotic disorder relative to HC, as measured by [^{18}F]fallypride PET.[62]

3.2.1.3. Cognitive task-induced DA release

Kasanova et al. (2018)[61] performed a [^{18}F]fallypride PET scan during a probabilistic reinforcement learning task in healthy individuals with a first-degree relative with

psychosis and HC. No group differences were reported in reward-induced DA release in the bilateral i) putamen, ii) CNC, or iii) VST.

3.2.1.4. Pharmacologically-induced DA release

Another way to induce DA release is by the administration of 2-Deoxy-D-Glucose (2-DG). Using this approach, Brunelin et al. (2010)[64] investigated the metabolic stress-induced DA release in healthy siblings of schizophrenic patients and HC. Only HC showed a significant decrease in striatal [^{11}C]raclopride binding (i.e., reflecting DA release induced by 2-DG) between pre- and post-2DG administration. This increase in DA release was not observed in the striatum of the first-degree relatives of schizophrenic patients or the nucleus accumbens (Nacc) of either group. Interestingly, in the first-degree relatives (but not controls), a lateralised (left > right) metabolic stress-response was apparent in the striatum and Nacc. Moreover, the level of Nacc asymmetry after exposure to stress was associated with positive schizotypy scores in both groups. In other words, the larger the level of asymmetry after stress induction, the greater the level of psychosis proneness. Pharmacologically-induced DA release has also been explored in a study by Kuepper et al. (2013).[66] They investigated the effect of delta-9-tetrahydrocannabinol (THC) on DA neurotransmission in healthy cannabis users, patients with a psychotic disorder and first-degree relatives of patients with a psychotic disorder (unrelated to the participating patients) by use of [^{18}F]fallypride PET. All participants had used cannabis at least once in the previous twelve months. THC administration resulted in significant DA release throughout the striatum of the first-degree relatives and psychotic patients. However, this was not the case for healthy cannabis users. Also, the extent of DA release was larger for the psychotic patients and first-degree relatives than for healthy cannabis users in the left CNC (corrected for gender, age, nicotine use, alcohol use, use of other drugs and medication, and frequency of cannabis use). No differences were reported between first-degree relatives and psychotic patients in this region. The main conclusion that can be drawn is that first-degree relatives of psychotic patients are likely to demonstrate differential sensitivity to multiple pharmacological compounds compared with HC.

3.2.1.5. DA $D_{2/3}$ receptor availability

First-degree relatives of patients with a diagnosis of a psychotic disorder did not differ significantly from HC with regard to [^{18}F]fallypride BP_{ND} in the left and right ventromedial PFC (vmPFC),[62] [^{11}C]raclopride binding in left and right i) putamen and ii) CNC,[63] or WS.[64] Furthermore, no correlation was found between genetic load and radiotracer binding.[63] However, Lee et al. (2008)[63] showed that unaffected family members of schizophrenic patients displayed a loss of asymmetry of the DA $D_{2/3}$ receptor availability in the putamen compared with HC, while HC had a higher DA $D_{2/3}$

receptor availability in the right compared with the left putamen. Furthermore, rightward asymmetry of DA $D_{2/3}$ receptor availability was not found in the CNC of either group.[63] Supplementary, Eisenstein et al. (2017)[68] reported no differences between siblings of schizophrenic patients, individuals with schizophrenia or schizoaffective disorder, and HC in [^{11}C]NMB BP_{ND} in the dorsal (i.e., putamen and CNC) or ventral (i.e., Nacc) areas of the striatum. Complementary to these findings, Hirvonen et a. (2005)[65] compared the [^{11}C]raclopride binding in the putamen, CNC, and thalamus between HC twins, monozygotic, and dizygotic healthy co-twins from pairs discordant for schizophrenia. A higher DA $D_{2/3}$ receptor availability was found in the CNC of monozygotic co-twins compared with dizygotic co-twins and HC twins. No group differences in DA $D_{2/3}$ receptor availability were reported between dizygotic co-twins and HC twins or in the putamen or thalamus. Although the quality evaluation of Brunelin et al. (2010)[64] resulted in the lowest mean OSQE score (i.e., four) of all included case-control studies, the results are in line with the findings of Lataster et al. (2014)[62] and Lee et al. (2008).[63] Furthermore, the mean OSQE score of Hirvonen et al. (2005)[65] did not seem aberrant compared to the scores of the other articles focussing on $D_{2/3}$ receptor availabilities in relatives (see eTable 3). Therefore, we can conclude that most studies did not find evidence for differences in DA $D_{2/3}$ receptor availability between first-degree relatives of psychotic patients and HC.

3.2.1.6. DA D_1-like receptor availability

In a subsequent article, Hirvonen et al. (2006)[67] used the [^{11}C]SCH 23390 radiotracer to examine the DA D_1-like receptor availability in the same cohort of twins as previously described by Hirvonen et al. (2005),[65] as well as in their affected schizophrenic probands (who had received chronic antipsychotic treatment). Compared with HC twins, monozygotic healthy co-twins showed increased DA D_1-like receptor binding in three areas: the mPFC, superior temporal gyrus, and angular gyrus. DA D_1-like receptor binding in dizygotic healthy co-twins was intermediate between monozygotic healthy co-twins and HC twins. Moreover, the DA D_1-like receptor binding in schizophrenic probands was reduced compared with their unaffected co-twins in a wide range of brain areas, including the striatum, parietal, temporal, and frontal cortices. This reduction of DA D_1-like receptor availability was associated with antipsychotic medication dose. Overall, increasing genetic risk for psychosis seems to be related to higher levels of DA D_1-like receptors. Finally, Hirvonen et al. (2006)[69] investigated the balance between striatal DA D_1-like and DA $D_{2/3}$ receptors in the same twin cohort and found no differences in DA D_1-like/$D_{2/3}$ ratios between healthy monozygotic co-twins, healthy dizygotic co-twins, and HC twins in the CNC or putamen.

3.2.2. Chromosomal abnormalities and genetic variations

3.2.2.1. sDSC

Dahoun et al. (2018)[71] investigated whether the DISC1 Ser704Cys single-nucleotide polymorphism (SNP; rs821616) influenced the sDSC by comparing [^{18}F]F-DOPA uptake between ser homozygotes (i.e., the group with an increased risk for psychosis) and cys homo- and heterozygotes. Significantly greater striatal [^{18}F]F-DOPA uptake was reported in ser homozygotes relative to cys homo- and heterozygotes. In addition, Rogdaki et al. (2021)[23] compared the sDSC of 22q11DS carriers, 22q11.2 duplication carriers (22q11DUP; which may be associated with a reduced risk of schizophrenia relative to the general population), and HC. The sDSC in the WS and all its functional subdivisions was higher in 22q11DS carriers compared with 22q11DUP carriers and HC. These findings remained significant after controlling for age and injected activity. Furthermore, across 22q11DS and 22q11DUP carriers, a significant relationship was found between WS [^{18}F]F-DOPA uptake and ratings on the positive symptom dimension of the CAARMS. This was not the case for the severity of anxiety and depressive symptoms. Although the quality assessment of Rogdaki et al. (2021)[23] resulted in a lower score compared to the quality assessment of Dahoun et al. (2018)[71] (i.e., mean OSQE score of six and nine, respectively; see eTable 3), the results of both studies point towards the same direction. In conclusion, several studies suggest that individuals with chromosomal abnormalities or genetic variations display increased sDSC compared with individuals without these alterations.

3.2.2.2. Cognitive task-induced DA release

Using [^{18}F]fallypride PET, participants in the study of van Duin et al. (2018)[74] were exposed to a probabilistic stimulus task (PSST) to investigate reinforcement learning-induced DA release in the striatum of 22q11DS and HC subjects. No significant group differences in PSST-induced DA release in the mean, left, and right i) VST, ii) putamen, or iii) CNC were reported (intelligence quotient [IQ] was included as a covariate in the analysis).

3.2.2.3. DA $D_{2/3}$ receptor availability

Besides the previously mentioned Ser704Cys SNP, other common polymorphisms can occur in the amino-acid sequence of the DISC1 protein, namely Leu607Phe (rs6675281) and Arg264Gln (rs3738401). The effects of any of these three DISC1 polymorphisms and striatal DA $D_{2/3}$ receptor availability were investigated by Dahoun et al. (2019)[73] by use of [^{11}C]-(+)-PHNO PET. No associations were reported between DISC1 polymorphisms and DA $D_{2/3}$ receptor availability in the WS, CNC and putamen. Furthermore, using [^{18}F]fallypride PET, van Duin et al. (2018)[74] investigated the availability of DA $D_{2/3}$ receptors in the striatum of 22q11DS individuals and HC (PET

baseline condition). No group differences with regard to [^{18}F]fallypride BP_{ND} in the mean, left, and right i) VST, ii) putamen, or iii) CNC were found (while adjusting for IQ). This is in agreement with the result of a SPECT study by Boot et al. (2010),[70] who also did not find striatal differences in [^{123}I]IBZM BP_{ND} between 22q11DS individuals and HC. However, in a subsequent article on the same cohort, although extended by two additional 22q11DS individuals, van Duin et al. (2020)[91] reported a lower DA $D_{2/3}$ receptor binding in the anterior cingulate gyrus of 22q11DS individuals relative to HC. This was not the case for other frontal regions, i.e., PFC (results did not survive correction for multiple testing), OFC, and ACC. Also, within the 22q11DS group, no association was found between [^{18}F]fallypride BP_{ND} in any of the frontal brain regions and PANSS scores. Interestingly, the same research group combined the data of two previously collected cohorts at clinical[46] and genetic high risk for developing psychosis[70] and compared their striatal DA functioning. Vingerhoets et al. (2018)[77] reported no significant effect of group (22q11DS, UHR, and HC) on striatal [^{123}I]IBZM BP_{ND} (while adjusting for age and gender). In sum, all included articles, except one,[91] indicate no alterations in striatal or extrastriatal DA $D_{2/3}$ receptor availabilities in individuals with chromosomal abnormalities (such as 22q11DS) or genetic variations (such as Ser704Cys SNP) relative to individuals without these alterations.

3.3. Environmental high-risk groups

Several environmental factors have been associated with an increased risk of developing psychosis.[18] Twenty-three studies addressed the dopaminergic system of these individuals.

3.3.1. Cannabis

3.3.1.1. sDSC

The [^{18}F]F-DOPA uptake was significantly lower in the WS, AST, and LST of regular (i.e., at least weekly) cannabis users who experienced psychotic-like symptoms in response to consuming cannabis compared with non-using controls.[78] These findings remained significant after covarying for the use of non-cannabis drugs and were not present in the SMST. When subdividing the cannabis group into individuals who met DSM-4 criteria for cannabis dependence or cannabis abuse and individuals who did not, Bloomfield et al. (2014)[78] reported a significantly lower sDSC in the WS of cannabis users who met the criteria for cannabis dependence/abuse compared with cannabis users who did not meet these criteria and compared with non-using controls. Analyses in the striatal subdivisions only revealed significantly lower sDSC in the AST of cannabis dependency/abuse group compared with non-dependence/non-abuse cannabis users. In addition, no sDSC differences in the WS were found between the non-dependence/non-abuse cannabis users and non-using controls. Within the cannabis

group, lower [18F]F-DOPA uptake in the WS, AST, and SMST was associated with higher levels of current cannabis use (i.e., less time to smoke one-eight ounce of cannabis). Moreover, younger age at first cannabis exposure was associated with lower sDSC in the WS and AST (after adjusting for current age). In sum, sDSC in the WS and AST seem to be lower in cannabis-using individuals compared with non-using HC, with larger alterations in heavier users.

3.3.1.2. Psychosocial stress-induced DA release

Individuals meeting DSM-4 criteria for cannabis dependence responded similarly to psychosocial stress while performing the MIST compared with HC,[48] as no differences were found in psychosocial stress-induced DA release in the WS, AST, LST, SMST, or SN. Conversely, a lower [11C]-(+)-PHNO ΔBP_{ND} was reported in the GP of cannabis users relative to HC. However, after excluding cannabis users from the analysis who reported having used cannabis less than 8 hours before the PET scans, the difference in tracer displacement did not remain significant.

3.3.1.3. Pharmacologically-induced DA release

Amphetamine induced less DA release in the WS, AST, and SMST of cannabis-dependent subjects than in HC.[81] A similar difference involved one extrastriatal region, namely the GP. Urban et al. (2012),[75] however, reported no differences in amphetamine-induced [11C]raclopride displacement between cannabis-dependent participants and HC in the WS, VST, AST, and SMST. Neither study reported a correlation between the severity of cannabis use of cannabis-dependent subjects and striatal amphetamine-induced DA release.[75,81] Furthermore, Urban et al. (2012)[75] did not find a significant correlation between amphetamine-induced DA release and the duration of cannabis abstinence. However, when controlling for current age, earlier age of onset was significantly associated with lower amphetamine-induced DA release in the AST and pre-DCA of cannabis-dependent subjects.[75] Stokes et al. (2009)[83] investigated the acute effect of another pharmacological challenge, namely THC consumption, on DA release in the striatum of healthy volunteers with a history of cannabis use (i.e., at least twenty times). No significant interaction was reported between previous cannabis exposure and the effect of THC on striatal [11C]raclopride BP_{ND}. Complementary, in a subsequent article, reporting on the same cohort, no association was found between THC-induced decrease of [11C]raclopride BP_{ND} in extrastriatal regions (i.e., right middle frontal gyrus, left superior frontal gyrus, and the left superior temporal gyrus) and lifetime frequency of cannabis use.[84] Finally, methylphenidate significantly decreased the [11C]raclopride BP_{ND} in the striatum of marijuana abusers (who met DSM-4 criteria for cannabis abuse or dependence) and HC.[80] Nevertheless, no group differences in methylphenidate-induced DA release were found in the striatum. Interestingly, larger methylphenidate-

induced BP_{ND} decreases were reported in the midbrain (centered in the SN encompassing the subthalamic nucleus) of marijuana abusers relative to HC. By using different pharmacological challenges, multiple studies investigated the subsequent effect on striatal and extrastriatal DA release. In conclusion, multiple studies find evidence for alterations in DA release in some brain regions, however, this has not been confirmed by others.

3.3.1.4. DAT

By use of [^{11}C]PE21 PET, Leroy et al. (2012)[82] investigated the DAT availability in striatal and extrastriatal brain regions of cannabis-dependent tobacco smokers (who met DSM-4 criteria for cannabis dependence), tobacco-dependent smokers (who met DSM-4 criteria for nicotine dependence), and healthy non-smokers. Both addicted groups showed a decreased DAT availability compared with non-smokers in the putamen and CNC. Whole-brain analysis extended these findings by also revealing reductions in [^{11}C]PE21 BP_{ND} in the VST, midbrain (consisting of the SN and VTA), grey and white matter of the cingulate gyrus, and several thalamic nuclei of cannabis- and tobacco-dependent smokers relative to non-smokers. No significant differences in DAT availability in any of the striatal or extrastriatal regions were found between cannabis- and tobacco-dependent smokers.[82] Furthermore, Leroy et al. (2012)[82] did not find significant correlations between [^{11}C]PE21 BP_{ND} and i) history use (i.e., joint years or pack years) or the age of onset, or ii) the number of joints and/or cigarettes smoked per day, in either cannabis-dependent tobacco or tobacco-dependent smokers. To conclude, both cannabis-dependent tobacco and tobacco-dependent smokers display lower DAT availabilities in multiple striatal and extrastriatal brain regions.

3.3.1.5. DA $D_{2/3}$ receptor availability

Region of interest and voxel-wise analyses revealed no differences in [^{11}C]raclopride BP_{ND} in the striatum (i.e., left and right pre-DCA, pre-DPU, post-commissural dorsal caudate [post-DCA], post-commissural dorsal putamen [post-DPU], and VST) between cannabis users (who consumed at least one joint per week in the previous month) and HC.[72] These findings were confirmed by Stokes et al. (2012)[76], who also reported no differences in DA $D_{2/3}$ receptor availability in the WS and its functional subdivisions between volunteers with a history of cannabis use (i.e., lifetime history of using cannabis $\geq$ 50 times) and HC (adjusted for current nicotine cigarette smoking status and age). Moreover, no correlations were found between [^{11}C]raclopride BP_{ND} in the WS, AST, SMST, or LST and i) frequency of lifetime cannabis use, ii) years of cannabis use, iii) age of first use, and iv) duration since last cannabis use.[76] However, a negative association was found between DA $D_{2/3}$ receptor availability and recent cannabis use per day in the cannabis group.[72] The lack of changes in DA $D_{2/3}$ receptor availability

has also been found in cannabis-dependent subjects across a broad range of regions.[75,79-81,92] Firstly, Sevy et al. (2008)[79] found no differences in [^{11}C]raclopride binding in the left and right i) CNC and ii) putamen of cannabis-dependent males who were abstinent for at least twelve weeks relative to HC. Secondly, marijuana abusers (who met DSM-4 criteria for cannabis abuse or dependence) did not differ from HC with regard to [^{11}C]raclopride BP_{ND} in the striatum (comprising the CNC, putamen, and VST)[80] or, in another study, with regard to [^{11}C]raclopride BP_{ND} in the putamen, GP, dorsal CNC, and VST.[92] Moreover, no DA $D_{2/3}$ receptor availability differences were reported between cannabis-dependent subjects and HC in the WS, VST, AST, and SMST,[75,81] nor in the GP, thalamus, or midbrain.[81] Urban et al. (2012)[75] did not find a significant correlation between [^{11}C]raclopride BP_{ND} and the severity of cannabis use or the time since last use. Finally, Mizrahi et al. (2013)[48] demonstrated an increased [^{11}C]-(+)-PHNO BP_{ND} in the WS, AST, LST, and SMST, but not in the GP and SN, of cannabis users meeting the DSM-4 criteria for cannabis dependence compared with HC. However, after excluding cannabis users from the analysis who reported having used cannabis less than 8 hours before the PET scan, no differences in BP_{ND} were observed. The result of the study quality assessment of Mizrahi et al. (2013)[48] was slightly above average compared to the other studies focussing on $D_{2/3}$ receptor availability in cannabis users (see eTable 3). Overall, striatal and extrastriatal DA $D_{2/3}$ receptor availability appears similar in cannabis users and HC.

3.3.2. Immigration

3.3.2.1. sDSC

Information on immigration status was available from various [^{18}F]F-DOPA PET studies[41,42,49] and analysed by Egerton et al. (2017).[85] An increased sDSC was found in the WS and SMST of immigrants compared with non-immigrants (adjusted for cannabis use).[85] Exploratory analysis revealed an elevated sDSC in second-generation immigrants compared with non-immigrants. Furthermore, no differences were found in sDSC between first-generation immigrants and second-generation immigrants or non-immigrants.

3.3.2.2. Psychosocial stress-induced DA release

Egerton et al. (2017)[85] also investigated whether stress-induced DA release was altered in immigrants compared with non-immigrants by use of data from previously performed [^{11}C]-(+)-PHNO PET studies.[21,34] Immigrants demonstrated an increased release of DA after exposure to psychosocial stress in the WS, AST, and LST relative to non-immigrants (adjusted for cannabis use). Exploratory analysis revealed an elevated striatal DA release in first-generation immigrants relative to non-immigrants, whereas no differences were found between second-generation immigrants and first-generation

immigrants or non-immigrants. Thus, the dopaminergic system of immigrants seems to be altered compared with non-immigrants (i.e., increased sDSC and psychosocial stress-induced DA release). However, differences between first- and second-generation immigrants may exist.

3.3.3. Childhood trauma / Adversity

3.3.3.1. sDSC

Childhood adversity was assessed in UHR and HC subjects by use of the Childhood Experience of Care and Abuse Questionnaire (CECA-Q). Subsequently, Egerton et al. (2016)[24] investigated the association between childhood adversity and previously collected [^{18}F]F-DOPA data.[21,34,93] Across UHR and HC subjects, sDSC in the WS and AST were significantly higher in subjects who had reported severe physical or sexual abuse during childhood compared with those who had not. This was also the case for subjects who had experienced more than two family arrangements (i.e., the number of different caregivers with each of whom the child lived for minimally one year) compared with those who had not. The results remained significant after controlling for age, gender, alcohol drinking or smoking status, and the use of cannabis, ecstasy, cocaine, amphetamine, or ketamine. Eight out of twenty-six subjects who reported physical or sexual abuse also reported multiple family arrangements. When both types of adverse events were added to the same model, only the effect of multiple family arrangements was significant. In addition, experiencing more than two family arrangements was also related to an elevated sDSC in the SMST. There was no evidence that the association between childhood adversity and DA functioning was different in UHR and HC subjects. Furthermore, no group differences in sDSC in the WS, AST, SMST, and LST were observed between UHR and HC subjects. Bloomfield et al. (2019)[94], in contrast, found a reduction of [^{18}F]F-DOPA uptake in the WS of individuals with high cumulative exposure to psychosocial adversity (i.e., exposed to minimally one childhood stressor and two adult stressors) compared with individuals with low exposure (i.e., not exposed to childhood stressors and no significant adverse events in the last six months). This reduction was also present in the AST and LST. However, after co-varying for current cigarette use, the sDSC group difference only remained significant in the LST. Thus, the findings of Egerton et al. (2016)[24] and Bloomfield et al. (2019)[94] are conflicting. However, methodological quality of both studies was similar (see eTable 3). In line with the findings of Bloomfield et al. (2019)[94], Schalbroeck et al. (2021)[95] reported the results of their exploratory post hoc analyses, which suggested a significant negative association between childhood trauma (measured with the Childhood Trauma Questionnaire; CTQ) and sDSC in the WS, AST, and SMST (adjusted for gender, age, scanner type, and smoking status) in HC. To summarise, results with regard to sDSC are inconsistent.

3.3.3.2. Psychosocial stress-induced DA release

Kasanova et al. (2016)[90] used [18F]fallypride PET to examine the relationship between early (ages 0–11) and late (ages 12–17) exposure to childhood trauma and spatial extent of psychosocial stress-induced DA release in patients with a non-affective psychotic disorder (NAPD) and HC. Interestingly, this relation was different in each group. Within the HC group, the spatial extent of the psychosocial stress-induced DA release in the mPFC was positively associated with early and late childhood trauma scores, while within the NAPD group these associations were not evident. Moreover, no significant relations were found between childhood trauma scores and psychosocial stress-induced DA release in either of the groups with regard to the vmPFC or dmPFC. Supplementary to the above-described results in the PFC, Pruessner et al. (2004)[38] investigated the effect of and low scores on the maternal care subscale of the Parental Bonding Index (PBI). The psychosocial stress task significantly reduced [11C]raclopride binding in the VST and putamen of subjects with low, but not high, self-reported early life maternal care. The previously described study of Soliman et al. (2008)[53] also reported that subjects with low maternal care scores showed the highest stress-induced striatal DA release across all participants (i.e., psychometric schizotypes and HC). In contrast, these findings were not replicated by Montgomery et al. (2006),[96] who reported no relation between maternal care scores and psychosocial-stress induced DA in either the dorsal striatum or VST of healthy volunteers. Mizrahi et al. (2012)[41] also did not find a relationship between psychosocial stress-induced [11C]-(+)-PHNO displacement in the striatum and early maternal care (measured by use of the PBI) in schizophrenic patients, UHR, or HC. In sum, early and late childhood trauma scores are positively associated with psychosocial stress-induced DA release in the mPFC of HC. This has not been found for other brain regions, such as the vmPFC and dmPFC. The study quality varied between the studies, however, no substantial differences were noted (see eTable 3). Therefore, we can conclude that while multiple studies found no association between striatal psychosocial stress-induced DA release and maternal care scores, others did find increased responses in subjects that reported low maternal care.

3.3.3.3. Pharmacologically-induced DA release

Administration of dexamphetamine resulted in a significant decrease of [11C]-(+)-PHNO BP_{ND} in the VST, AST, and SMST of healthy volunteers and an increase of psychotic symptoms (i.e., PANSS positive items).[88] Dahoun et al. (2019)[88] also reported a negative association between ventral striatal DA release and childhood trauma load (measured by use of the CTQ). This is not in line with the prediction that childhood trauma sensitises the DA system.[97] The findings of Dahoun et al. (2019)[88] may be explained by lower levels of perceived stress (i.e., the degree to which events are evaluated as stressful) in individuals with high CTQ scores. Furthermore, the interaction

between childhood trauma and dexamphetamine-induced DA release in the VST (not evident for the AST or SMST) predicted dexamphetamine-induced positive psychotic symptoms (not evident for non-psychotic psychopathology).[88] This relationship was specific to DA release capacity and was not significant for pre-dexamphetamine/baseline DA $D_{2/3}$ receptor availability in the VST. The subsequent mediation analysis revealed no indirect effect of childhood trauma on dexamphetamine-induced positive psychotic symptoms through dexamphetamine-induced DA release, i.e., childhood trauma did not lead to sensitization of dexamphetamine-induced DA release. Oswald et al. (2014)[87] also reported a significant amphetamine-induced DA release in the VST of healthy individuals. Additionally, a positive relation was observed between Early Trauma Inventory (ETI) scores and amphetamine-induced DA release in the VST. This indicates that subjects who report more adverse events during childhood have larger DA responses to amphetamine in the VST. These findings turned out to be specific for the VST, while no significant associations were reported between ETI scores and DA release in the i) anterior CNC, ii) posterior CNC, iii) anterior putamen, or iv) posterior putamen. Interestingly, the relation between ETI scores and amphetamine-induced ventral striatal DA release was mediated by scores on the Perceived Stress Scale. As the study quality evaluation of Dahoun et al. (2019)[88] and Oswald et al. (2014)[87] resulted in similar scores (see eTable 3), we can summarize that, both, a negative and a positive association have been observed between childhood trauma and amphetamine-induced DA release in the VST.

3.3.3.4. DAT

Hoexter et al. (2012)[89] evaluated the DAT availability in the striatum of victims of violence who did or did not (resilient group) develop a post-traumatic stress disorder (PTSD). Resilient subjects demonstrated significantly lower [^{99m}Tc]TRODAT-1 binding in the left, right, and mean striatum compared with PTSD patients.

3.3.3.5. DA $D_{2/3}$ receptor availability

Dahoun et al. (2019)[88] did not find a relation between baseline DA $D_{2/3}$ receptor availability and childhood trauma in the VST of healthy volunteers. In contrast, Oswald et al. (2014)[87] reported an association between [^{11}C]raclopride BP_{ND} in the VST and scores on the ETI. This relation was different in females and males, with a non-significant slightly negative association in females and a significant positive association in males. Exploratory analyses also revealed a positive correlation between ETI scores and DA $D_{2/3}$ receptor availability in the anterior putamen and anterior CNC of males. Overall, VST DA $D_{2/3}$ receptor availability seems unrelated to childhood trauma. However, gender differences may exist.

3.3.4. Severe hearing impairment

3.3.4.1. Pharmacologically-induced DA release

Hearing impairment is another risk factor for psychotic disorders.[98] Gevonden et al. (2014)[86] examined with SPECT the dopaminergic system of individuals with a severe hearing impairment. They found a significant effect of severe hearing impairment on dexamphetamine-induced DA release in the striatum, with a larger DA release in individuals with a severe hearing impairment than in HC (adjusted for age and tobacco smoking).

3.3.4.2. DA $D_{2/3}$ receptor availability

No group differences in striatal [^{123}I]IBZM BP_{ND} were found between subjects with a severe hearing impairment and HC.[86]

3.4. Risk of bias assessment

The risk of bias of the included articles was assessed by use of the OSQE (see eTable 3 and eTable 4). The mean OSQE total score was 7.66 (SD: 1.25; range: 4.00–10.00) for case-control studies and 4.40 (SD: 1.07; range: 3.00–6.00) for cross-sectional studies. The majority of the included articles did not provide information with regard to non-response and missing data. Therefore, almost all articles received no star on items 1, 9, 10, and 11. This highlights the need to accurately and precisely report the performed procedures. Since, the raters tried to avoid making assumptions about the presence/absence of particular information (i.e., if the information was not mentioned in the article, no star could be appointed), the scoring can be considered conservative.

4. Discussion

To our knowledge, the current systematic review is the first to address dopaminergic alterations, as demonstrated by imaging studies, in populations at increased risk of developing psychosis (i.e., clinical, genetic, and environmental high-risk groups) and relate the dopaminergic system of these individuals to the dopaminergic system of HC and schizophrenic patients. Here we will first discuss the main evidence per high-risk group, followed by a general section.

4.1. Clinical high risk

The current findings extend the understanding of the dopaminergic system of clinical high-risk individuals by showing that:

1) Individuals who meet UHR criteria for being at clinical high-risk of psychosis are likely to show an increased sDSC in the WS and AST (especially those UHR subjects who develop psychosis) and elevated striatal psychosocial stress-

induced DA release compared with HC. Furthermore, in this clinical high-risk group striatal and extrastriatal DA $D_{2/3}$ receptor availabilities seem not to be altered compared with HC.

2) Clinical high-risk individuals meetings DSM-4 criteria for SPD are likely to exhibit an increased sDSC in the WS, AST, and SMST relative to HC, as well as, no changes with respect to striatal DA $D_{2/3}$ or prefrontal and striatal DA D_1-like receptor availabilities.

3) Striatal DA $D_{2/3}$ receptor levels and psychosocial stress-induced DA release seem not to be altered in healthy individuals with schizotypal personalities. However, striatal amphetamine-induced DA release appears to be related to schizotypal traits in healthy individuals.

4.1.1. UHR individuals

Multiple studies hint toward elevations in sDSC in the WS and AST, especially in subjects who subsequently develop psychosis, as well as, elevated striatal psychosocial stress-induced DA release in UHR relative to HC. These findings are in line with findings in schizophrenic patients. First of all, a meta-analysis demonstrated increased presynaptic DA functioning in the WS, AST, and SMST of schizophrenic patients compared with HC.[99] Secondly, striatal DA release was significantly higher in schizophrenic patients than controls (studies using amphetamine or psychosocial stress as stressors were combined).[20] This agreement of findings supports their validity.

In contrast, a recently published meta-analysis did not find evidence of significant differences between clinical high-risk individuals and controls in striatal presynaptic dopaminergic functioning.[100] However, since this group difference approached significance ($p = 0.07$), it is too early to rule out the possibility of significant differences between groups with regard to presynaptic dopaminergic functioning. In addition, McCutcheon et al. (2021)[100] combined studies investigating sDSC (assessed by use of [^{18}F]F-DOPA PET) and DA release capacity (assessed by use of [^{123}I]IBZM, [^{11}C]-(+)-PHNO, and [^{11}C]raclopride SPECT/PET in combination with a pharmacological or behavioural challenge). As the authors note, although various aspects of dopaminergic functioning are related, there is evidence that these paradigms capture separate aspects of dopaminergic functioning.[101] Additionally, the use of different radiotracers and the combination of multiple at-risk groups (i.e., UHR and SPD) make it challenging to interpret the outcome of their meta-analysis, as well as, to draw conclusions for separate at-risk groups and facets of the dopaminergic system.

Moreover, inconsistent study results might be explained by various factors. For example, the described alterations with regard to DA release in UHR subjects may be region and challenge specific, as Girgis et al. (2019)[45] did only report a greater methylphenidate-induced ΔBP_{ND} for the VST compared to HC, which did not extend

to other striatal subregions or extrastriatal regions. In addition, Bloemen et al. (2013)[46] did not report a significant difference in AMPT-induced DA depletion in the striatum between UHR and HC. Furthermore, changes in presynaptic DA functioning in UHR were not confirmed by other researchers.[33,36] These discrepancies may be due to limited power to detect group differences, to changes in the population referred to early detection services over time (i.e., in recent cohorts subjects are referred earlier), to different UHR criteria (i.e., the occurrence of a brief limited intermittent psychotic episode is closer related to a psychotic disorder than the presence of attenuated psychotic symptoms),[45] or due to the various imaging techniques used (i.e., the first SPECT studies could only address the radiotracer binding in the WS, while modern PET studies are now also able to investigate subdivisions of the striatum). Subsequently, DA dysregulation may initially occur in the VST and may propagate later to other adjacent regions of the striatum as the at-risk individual progresses towards a first-psychotic episode.[45] This is in line with Howes et al. (2011)[93] who showed an increase of [^{18}F]F-DOPA in the AST and SMST as UHR individuals transitioned to schizophrenia. In addition, several other studies found changes in the dopaminergic system of only those UHR subjects who subsequently transitioned to a first-episode psychosis compared with HC,[8,33,37] although, this has not been confirmed by others.[36,45] This inconsistency between studies may be explained by the fact that UHR subjects continue to be at risk for psychosis up to 10 years after the initial referral,[102] so a longer follow-up period may be required to elucidate group differences.

Alternatively, the lack of evidence may also be declared by the heterogeneous characteristics of the UHR group. It is likely that the whole UHR group can be stratified into multiple subgroups, with varying risks to develop psychosis, transition rates, and underlying neurobiology. UHR subjects with the highest risk of developing psychosis may exhibit the largest dopaminergic alterations compared with HC, while UHR subjects with increased risks of developing psychosis compared with the general population but lower risks than other UHR subjects may have subtle or no dopaminergic deviations compared with HC. This is supported by several studies that reported associations between dopaminergic outcomes and psychopathology,[21,36,46] as well as, the meta-analysis of McCutcheon et al. (2021)[100] who reported that the difference in variability of presynaptic dopaminergic functioning for clinical high-risk individuals compared to controls almost reached significance ($p = 0.06$).

The absence of group (UHR versus HC) differences with regard to DA $D_{2/3}$ receptor availability is in line with findings of recent meta-analyses, where striatal DA $D_{2/3}$ receptor binding is unaltered in clinical high-risk individuals[100] and schizophrenic patients compared with HC.[20] Another meta-analysis of seven post-mortem studies showed that DA D_2 receptor densities were increased in schizophrenic patients compared with HC.[103] The differences between *in vivo* and post-mortem results may be

explained by the finding that baseline occupancy of striatal DA D_2 receptors by DA is also increased in schizophrenia.[104] As PET and SPECT radiotracers cannot bind to DA receptors that are occupied by endogenous DA, DA D_2 receptor differences can be masked by simultaneously altered endogenous DA levels.

One article did find a higher DA $D_{2/3}$ receptor availability in UHR compared with HC in the dlPFC, ACC, and mPFC.[44] This could be related to the heterogeneity inherent to the UHR population. In line with the post-mortem results, Brugger et al. (2020)[20] did find evidence for increased variability of striatal DA $D_{2/3}$ receptor availability in patients with schizophrenia compared with HC. This suggests that a subgroup of schizophrenic patients may exist in whom striatal DA $D_{2/3}$ receptor availability does differ from healthy subjects. This may also be the case for the UHR group. However, McCutcheon et al. (2021)[100] did not find significant differences in DA $D_{2/3}$ receptor variability between clinical high-risk individuals and controls.

The results of multiple publications point toward predominantly presynaptic dopaminergic alterations in UHR individuals. However, this finding has not been replicated by a recently published meta-analysis[100] and as some of these topics have only been addressed within a single research setting (i.e., sDSC in UHR subjects has mainly been studied by researchers of King's College London), replication (in a different institute) would be preferable.

4.1.2. Schizotypal personality disorder

Our finding of an increased sDSC in the WS, AST, and SMST of clinical high-risk individuals meeting DSM-4 criteria for SPD relative to HC is consistent with evidence that individuals with SPD demonstrate increased striatal DA release to amphetamine compared with HC subjects.[50] This elevation is similar to that observed in remitted schizophrenia and at an intermediate level between acute patients with schizophrenia and HC.[50] However, this finding has not been confirmed by others.[51] Whereas a trend level association was found between amphetamine-induced DA release in the VST and the severity of psychotic-like symptoms, the discrepant findings may be explained by variability in schizotypal symptom severity.[51] Future research that evaluates subgroups of SPD patients based on clinical presentation may further elucidate altered striatal DA functioning in these patients.

Moreover, we found no evidence for alterations in SPD patients compared with HC in terms of striatal DA $D_{2/3}$[50,51] and striatal or frontal DA D_1-like receptor availabilities.[52] This is partly in agreement with findings of unaltered striatal DA $D_{2/3}$ receptor availabilities in patients with schizophrenia[20] and clinical high-risk subjects meeting UHR criteria.[42,46,100] However, a previous study reported a significantly higher DA D_1-like receptor availability in the dlPFC of schizophrenic patients compared with HC.[105] It is important to note, that the increased PFC DA D_1-like receptor availability

was only confirmed in drug-naïve schizophrenic patients, but not in patients with a history of antipsychotic medication.[106] Nevertheless, it may be the case that alterations in DA D_1-like receptor availability only occur to a minor extent in SPD compared with schizophrenic patients or that they are only present in a subgroup of SPD subjects.

4.1.3. Schizotypal traits

The finding of a relation between striatal amphetamine-induced DA release and schizotypal traits in healthy individuals is consistent with findings in schizophrenic and SPD patients, which show exaggerated striatal amphetamine-induced DA release.[50,107,108] Overall, presynaptic aspects of the dopaminergic system, such as sDSC and DA release, seem to be elevated in the striatum of some individuals with a clinical high-risk for developing psychosis compared with healthy volunteers, while postsynaptic DA receptor availabilities in the striatum seem to be unaltered.

4.2. Genetic high risk

The current findings extend the understanding of the dopaminergic system of genetic high-risk individuals by showing that:

1) Most studies do not find evidence for differences in DA $D_{2/3}$ receptor availability in cortical and striatal areas between first-degree relatives of psychotic patients and HC. Furthermore, sDSC results are inconsistent in this genetic high-risk group.
2) Individuals with chromosomal abnormalities or genetic variations (such as 22q11DS or polymorphisms influencing the DISC1 protein) displayed an increased sDSC and no alterations in DA $D_{2/3}$ receptor availability in the striatum or extrastriatal regions compared with individuals without these alterations.

4.2.1. Relatives

Our finding of unaltered DA $D_{2/3}$ receptor availability in first-degree relatives of psychotic patients relative to HC is consistent with previous findings in schizophrenic patients and individuals with an increased genetic risk for schizophrenia. Brugger et al. (2020)[20] and McCutcheon et al. (2021)[100] found no differences in striatal DA $D_{2/3}$ receptor availability between patients or genetic high-risk subjects and controls, respectively. One study, however, reported contrasting results. Hirvonen et al. (2005)[65] found a higher DA $D_{2/3}$ receptor availability in the CNC of monozygotic unaffected co-twins from pairs discordant for schizophrenia compared with dizygotic co-twins and HC twins. These conflicting results may be caused by differences in the genetic relatedness between the affected family member and their monozygotic co-twins (about 100%) or dizygotic co-twins, parents, and children (about 50%). It may be the case that

only subjects who are genetically highly similar to a schizophrenic patient will exhibit DA $D_{2/3}$ receptor alterations compared with HC, while subjects who are genetically less similar to a schizophrenic patient will exhibit subtle or no DA $D_{2/3}$ receptor deviations compared with HC. Furthermore, dissimilarities in the age of the study populations have also been considered a reason for the discrepant outcomes.[63] Noteworthy, many of the participants of Hirvonen et al. (2005)[65] exceeded the peak age of onset for psychosis.

Additionally, an inconsistent pattern of results was obtained with regard to sDSC in first-degree relatives of patients with psychosis and HC. No alterations,[60] as well as, an elevated sDSC has been reported.[22] This may be explained by differences in symptom levels between the unaffected co-twins (who were asymptomatic) and first-degree relatives of schizophrenic patients (who had noticeable symptom levels). In addition, the patients with schizophrenia, who were not acutely unwell, also showed no elevation of sDSC.[60] This suggests that striatal hyperdopaminergia may be a state instead of a trait marker.

4.2.2. Chromosomal abnormalities and genetic variations

The result of elevated sDSC in individuals with chromosomal abnormalities or genetic variations is consistent with preclinical research which shows that the ser 704 DISC1 variant is associated with increased activity of ERK1/2 (which enhances the phosphorylation of tyrosine, the rate-limiting enzyme involved in the synthesis of dopamine).[109,110] However, McCutcheon et al. (2021)[100] did not find evidence of significant differences between genetic high-risk individuals and controls in striatal presynaptic dopaminergic functioning. However, multiple at-risk groups (i.e., first-degree relatives of psychotic patients and 22q11DS individuals) and aspects of dopaminergic functioning (i.e., sDSC and striatal DA release) were combined. This was reflected in a substantial between-study inconsistency (I^2 =77%).

Comparable to findings in schizophrenic patients[20] and the clinical high-risk group, striatal DA $D_{2/3}$ receptor availability was not altered in groups with chromosomal abnormalities or genetic variations compared with HC. van Duin et al. (2020)[91] did find a significantly lower DA $D_{2/3}$ receptor binding in the anterior cingulate gyrus of 22q11DS individuals relative to HC (but not in the PFC, OFC, and ACC; after correction for multiple testing), but this reduction is expected, as one of the genes located on the deleted region of chromosome 22q11.2, the catechol-O-methyltransferase (*COMT*) gene, codes for enzymes that are responsible for the catabolism of extracellular DA.[111] Higher DA concentrations in the synaptic cleft of 22q11DS individuals (due to less catabolism of DA), likely result in lower DA $D_{2/3}$ receptor availability due to competition and/or downregulation of these DA receptors.[91] As *COMT* haploinsufficiency is thought to mainly influence frontal DA,[112]

this may explain the differences between striatal and extrastriatal brain regions. Furthermore, the lack of group differences in other frontal regions of interest in van Duin et al. (2020)[91] may be accounted for by a lack of power.

Overall, sDSC seems to be elevated in some individuals with genetic high-risk for developing psychosis compared with healthy volunteers, while postsynaptic striatal DA $D_{2/3}$ receptor availabilities seem to be unaltered. Conclusive evidence with regard to alterations in other aspects of the dopaminergic system in this risk group is currently lacking and future research, especially those studies that focus on family members of patients with schizophrenia, should confirm the genetic risk status of the study participants.

4.3. Environmental high risk

The current findings extend the understanding of the dopaminergic system of environmental high-risk individuals by showing that:

1) In cannabis-using individuals, sDSC is decreased compared with non-using HC, with a greater decrease in heavier users. Moreover, striatal and extrastriatal DA $D_{2/3}$ receptor availability appears similar in cannabis users and HC.
2) The dopaminergic system of immigrants seems to be altered compared with that of non-immigrants (i.e., increased sDSC and psychosocial stress-induced DA release in the striatum), but differences between first- and second-generation immigrants may exist.
3) VST DA $D_{2/3}$ receptor availability appears unrelated to childhood trauma. However, gender differences may exist. Results concerning other parts of the dopaminergic system (i.e., sDSC, pharmacologically- and psychosocial stress-induced DA release) are less consistent.
4) Amphetamine-induced DA release in the striatum is significantly larger in individuals with a severe hearing impairment relative to HC. Individuals with a severe hearing impairment displayed no differences with regard to striatal DA $D_{2/3}$ receptor availability compared with HC.

4.3.1. Cannabis

Our finding that sDSC is lower in current cannabis users than nonusers is neither consistent with the hypothesis that increased sDSC underlies the association between cannabis and risk of psychosis, nor with the results reported for clinical and genetic high-risk groups. However, a biphasic dose-dependent dopamine response to THC may exist.[113] Within the cannabis group, lower [^{18}F]F-DOPA uptake in the WS, AST, and SMST was associated with higher levels of current cannabis use, suggesting greater alterations in heavier users.[78] Furthermore, a blunted dopaminergic system has also been

reported with other drugs of addiction.[114] Previous data in schizophrenic patients with comorbid dependence (including cannabis dependence) also showed that their striatal DA release was significantly blunted compared to patients without comorbid substance abuse, whereas the positive correlation between DA release and positive symptoms was preserved in both groups.[52] The lack of striatal and extrastriatal DA $D_{2/3}$ receptor group differences between cannabis users and healthy individuals is in line with previous findings in other high-risk groups[45,46,70,100] and schizophrenic patients.[20]

4.3.2. Immigration, childhood trauma, and severe hearing impairment

Previously published studies suggest that discrimination, victimization, social defeat, social isolation, and growing up in an urban environment may contribute to an increased risk of developing psychosis.[115-117] DA dysregulation has been suggested to be the link between social defeat and psychosis.[118] A study in rats has reported that experiencing social defeat stress can lead to striatal DA elevation.[119] This is in line with findings of increased sDSC and psychosocial stress-induced DA release in the striatum of immigrants compared with non-immigrants and increased striatal amphetamine-induced DA release in individuals with a severe hearing impairment relative to HC. DA dysregulation has also been reported in individuals who experienced childhood adversity. Egerton et al. (2016)[24] reported that sDSC in the WS and AST was significantly higher in subjects who reported severe physical, sexual abuse, or more than two family arrangements during childhood compared with those who had not. However, Bloomfield et al. (2019)[94] found a reduction of [^{18}F]F-DOPA uptake in the WS of individuals with high cumulative exposure to psychosocial adversity compared with individuals with low exposure to adversity. This is in line with results from animal studies where subcortical DA transmission is blunted in reaction to multiple stressors in adulthood.[120,121] Possibly, exposure to moderate stressors causes an initial sensitisation of the dopaminergic system, whereas repeated exposure to severe types of stress results in a subsequent down-regulation of the dopaminergic system.[94] Inconsistencies between the studies may also be clarified by exposure to different types of stressors or study populations. Egerton et al. (2016)[24] only investigated the effect of childhood factors, while Bloomfield et al. (2019)[94] also investigated adult factors. Furthermore, the cohort of Egerton et al. (2016)[24] consisted of UHR and HC, while the cohort of Bloomfield et al. (2019)[94] excluded individuals with an at-risk mental state. Lastly, DA $D_{2/3}$ receptor levels seem to be unaltered in individuals who experienced childhood trauma or in subjects with severe hearing impairment in the VST and striatum, respectively. This corresponds to the findings in other high-risk groups[45,46,70,100] and schizophrenic patients.[20]

Overall, sDSC findings in the environmental high-risk group are inconsistent. As with the clinical and genetic high risk groups it seems likely that the whole

environmental high group can be stratified into multiple subgroups, with varying risks to develop psychosis, transition rates, and underlying neurobiology. In contrast to the increased sDSC reported in some clinical and genetic high-risk groups, the sDSC in cannabis-using subjects seems to be decreased compared to HC. Furthermore, the association between sDSC and other environmental risk factors (such as childhood trauma) seems to depend on the type of exposure. Finally, striatal and extrastriatal DA $D_{2/3}$ receptor availabilities seem not to be altered in the environmental high-risk group.

4.4. Limitations

There are several limitations to the current study. First, some aspects of the dopaminergic system were only investigated by a limited amount of studies (e.g., cognitive task-induced DA release and neuromelanin concentrations). Therefore, these findings should be interpreted with caution and a firm conclusion cannot be drawn. Second, for most studies, no follow-up information was available, so differences between at-risk individuals who later developed psychosis and those who did not could only be addressed to a limited extent. In the future, more longitudinal studies are warranted. In addition to the previous point, the whole at-risk population may be stratified into multiple subgroups, with varying risks to develop psychosis and transition rates, as well as, underlying neurobiology. Besides DA, other non-dopaminergic systems, such as glutamatergic and GABAergic systems, may be altered in individuals with an increased risk of developing psychosis. To investigate this hypothesis, studies that use multimodal imaging and large sample sizes are necessary. Third, variation in the quality of studies is a potential source of bias. The risk of bias assessment revealed some variation in study quality. Fourth, another limitation is the use of different PET/SPECT radiotracers (except for the studies addressing sDSC) to address the same part of the dopaminergic system. This may induce discrepancies between study results. Fifth, we did not include clinical populations such as individuals diagnosed with PTSD or autism spectrum disorder. Although these patients also have an increased risk of developing psychosis, addressing these clinical groups is behind the scope of this review. Sixth, some individuals may belong to more at-risk groups. For example, some studies included cannabis-using UHR subjects or immigrants who met UHR criteria. Often information about family history of psychotic disorders, cannabis use, migration, or childhood trauma is not collected, and therefore stratification in homogenous subgroups may be difficult. Furthermore, relatives of schizophrenic patients may be part of the UHR group (i.e., genetic risk and deterioration syndrome group) if there is significant functional decline. This was, however, often not reported in the included studies focussing on the dopaminergic system in first-degree relatives of psychotic patients. Future studies should avoid this. Finally, although narrative reviews are important to describe trends in the literature, this qualitative approach does not make

use of an objective quantitative method, such as a meta-analysis, to analyse and summarize the findings of the individual studies. However, as this systematic review discussed a wide range of populations (i.e., three high-risk groups consisting of multiple subgroups) and study outcomes (i.e., different aspects of the dopaminergic system measured with various PET, SPECT, and NM-MRI imaging techniques), combining the individual studies would add substantial heterogeneity to a meta-analysis, and therefore hampers the interpretation of the results. Thus, it is important to extend current research findings, so future research can combine data of homogenous subgroups by use of a quantitative approach.

4.5. Implications and future directions

Our findings suggest increased sDSC in some clinical and genetic high-risk groups and unaltered DA $D_{2/3}$ receptor availability in clinical, genetic, and environmental high-risk groups. Findings concerning sDSC in the environmental high-risk group were less consistent, with a decreased sDSC in cannabis-using compared to healthy individuals. On the question whether differences exist between at-risk individuals and HC with regard to other parts of the dopaminergic system, our study presents a mixed picture. More longitudinal studies with larger samples, conducted over multiple years, which measure the dopaminergic system in several ways (i.e., during rest and following pharmacological, behavioural, or cognitive challenges), are needed to further examine these aspects of the dopaminergic system. When larger samples are collected, it will also be possible to create homogenous subgroups and study their risk of developing psychosis, transition rate, and underlying neurobiology.

5. Conclusions

To our knowledge, the present systematic review is the first to address the dopaminergic systems of individuals with a clinical, genetic, or environmental high risk of developing psychosis. This review supports the hypothesis that some DA abnormalities already occur before clinical, genetic, and environmental high-risk groups transition to psychosis. Our findings suggest that the detection of a hyperdopaminergic state in case of some clinical and genetic high-risk groups and hypodopaminergic state in case of the cannabis-using environmental high-risk group, as indexed by molecular imaging, may facilitate early detection and intervention of psychosis.

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7. Author contributions

CvH, EvdG, JB, JPS, and TvA conceived and designed the study. CvH and TvA designed the search strategy. CvH and DK (see acknowledgements) did the literature search, selected the studies, and extracted the relevant information. CvH and MD/DK/AW performed the quality assessment of included articles with support from MD. CvH synthesized the data and wrote the manuscript with support from MD, EvdG, JB, JPS, and TvA. All the authors critically reviewed the manuscript for intellectual content. All authors approved the final version of the manuscript for publication. TvA and JPS supervised the project.

8. Declarations of interest

None.

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Appendix to Chapter

Supplementary information can be found in the eBook (pages 85-96):

Chapter

Striatal dopamine transporter in individuals with chromosome 22q11.2 copy number variants: an [^{123}I]FP-CIT SPECT study

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Chapter

The relationships between dopaminergic, glutamatergic, and cognitive functioning in 22q11.2 deletion syndrome: a cross-sectional, multimodal ^{1}H-MRS and [^{18}F]fallypride PET Study

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Abstract

Background

Individuals with 22q11.2 deletion syndrome (22q11DS) are at increased risk of developing psychosis and cognitive impairments, which may be related to dopaminergic and glutamatergic abnormalities. Therefore, in this exploratory study, we examined the association between dopaminergic and glutamatergic functioning in 22q11DS. Additionally, the associations between glutamatergic functioning and brain volumes in 22q11DS and healthy controls (HC), as well as those between dopaminergic and cognitive functioning in 22q11DS, were also examined.

Methods

In this cross-sectional, multimodal imaging study, glutamate, glutamine, and their combined concentration (Glx) were assessed in the anterior cingulate cortex (ACC) and striatum in 17 22q11DS patients and 20 HC using 7T proton magnetic resonance spectroscopy. Ten 22q11DS patients also underwent [^{18}F]fallypride positron emission tomography to measure dopamine $D_{2/3}$ receptor ($D_{2/3}R$) availability in the ACC and striatum. Cognitive performance was assessed with the Cambridge Neuropsychological Test Automated Battery.

Results

No significant associations were found between ACC or striatal (1) glutamate, glutamine, or Glx concentrations and (2) $D_{2/3}R$ availability. In HC but not in 22q11DS patients, we found a significant relationship between ACC volume and ACC glutamate, glutamine, and Glx concentration. In addition, some aspects of cognitive functioning were significantly associated with $D_{2/3}R$ availability in 22q11DS. However, none of the associations remained significant after Bonferroni correction.

Conclusions

Although our results did not reach statistical significance, our findings suggest an association between glutamatergic functioning and brain volume in HC but not in 22q11DS. Additionally, $D_{2/3}R$ availability seems to be related to cognitive functioning in 22q11DS. Studies in larger samples are needed to further elucidate our findings.

1. Introduction

22q11.2 deletion syndrome (22q11DS), with a prevalence of 1 in 2000–4000 births, is a relatively common genetic disorder that is characterized by a microdeletion on chromosome 22 locus q11.2.[1] The typically deleted region contains approximately 90 genes.[2] Half of these are protein-coding genes, most of which are expressed in the brain.[2] The phenotypic expression of 22q11DS is highly heterogeneous and includes, among others, palatal anomalies, hypocalcemia, and congenital heart diseases.[3] Furthermore, the lifetime risk of developing a psychotic disorder for individuals with 22q11DS is 20–40%,[4] compared to 1–3% in the general population.[5] Individuals with 22q11DS often experience cognitive impairments, which can decline further with age.[6] Moreover, the cognitive decline is steeper in individuals with 22q11DS who develop a psychotic disorder.[6,7]

Two of the geneswithin the deleted region in 22q11DS are the catechol-O-methyltransferase (*COMT*) and proline dehydrogenase (*PRODH*) genes. The *COMT* gene encodes the *COMT* enzyme, which catabolizes extracellular dopamine. Dopamine levels in frontal brain regions are especially thought to be affected by the haploinsufficiency of the *COMT* gene.[8] Previous imaging studies have investigated dopaminergic functioning in subjects with 22q11DS and reported increased striatal dopamine synthesis capacity,[9] as well as reduced dopamine $D_{2/3}$ receptor ($D_{2/3}R$) binding in frontal brain areas of individuals with 22q11DS compared to healthy controls.[10]

The *PRODH* gene encodes the *PRODH* enzyme, which plays a role in the degradation of proline. The degradation of proline generates glutamate. Proline and glutamate can both, among other functions, activate the glutamatergic N-methyl-D-aspartate (NMDA) receptor.[11,12] It has been hypothesized that reduced *PRODH* enzyme activity in 22q11DS due to haploinsufficiency of the *PRODH* gene results in elevated proline levels.[13] Hyperprolinemia is a common finding in patients with 22q11DS.[13-15] Elevated proline levels may cause elevated activation of the NMDA receptor and excessive glutamate release.[11,16] Excessive glutamate levels are neurotoxic and can lead to neuronal injury and subsequent cell death.[17] Patients with excitotoxic damage are expected to have worse outcomes (i.e., more neurodegeneration, cognitive deficits, and negative symptoms) than patients without excitotoxic damage.[18] Due to *PRODH* haploinsufficiency, glutamate neuroexcitotoxicity may occur more frequently in 22q11DS relative to healthy individuals, which might explain the reduced cortical brain volumes reported in these patients.[19] Nevertheless, recent studies did not reveal significant alterations in glutamatergic functioning, as assessed by proton magnetic resonance spectroscopy (^{1}H-MRS), in the ACC or the striatum of patients with 22q11DS compared to healthy controls.[20,21] However, increased hippocampal glutamate

and Glx (glutamate and glutamine combined) concentrations were found in 22q11DS patients who developed schizophrenia compared to 22q11DS patients who did not.[22]

In schizophrenia and corresponding at-risk populations, increased striatal dopamine synthesis capacity has been a well-replicated finding.[23-26] However, in recent years, additional theories have been posited, suggesting that disrupted cortical glutamatergic functioning might underlie these striatal dopaminergic alterations in schizophrenia.[27] Preclinical studies, as well as *in vivo* studies, have demonstrated a relationship between dopaminergic and glutamatergic functioning. For example, the administration of ketamine, which blocks the NMDA receptors on y-aminobutyric acid (GABA)-ergic interneurons, resulting in the disinhibition of glutamatergic neurons and increased striatal dopamine levels in rodents.[28] Furthermore, positron emission tomography (PET) studies showed that the administration of ketamine increased synaptic dopamine levels in the striatum of healthy human volunteers.[29,30] Finally, a multimodal [^{18}F]F-DOPA PET and ^{1}H-MRS imaging study reported an inverse relation between glutamate concentration in the ACC and striatal dopamine synthesis capacity in patients with psychosis.[31]

In summary, possible alterations in dopaminergic and glutamatergic systems in individuals with 22q11DS might explain the increased risk of developing a psychotic disorder, as well as the increased prevalence of cognitive impairments in these patients. Although previous studies have examined dopaminergic[9,10,32] and glutamatergic functioning[20,21] in individuals with 22q11DS, to the best of our knowledge, no study has examined whether cortical and striatal glutamatergic and dopaminergic measures are correlated in individuals with 22q11DS. Therefore, we investigated glutamate, glutamine, and Glx concentrations in the ACC and striatum in relation to frontal and striatal dopamine $D_{2/3}$R availability in individuals with 22q11DS using ^{1}H-MRS and [18F]fallypride PET, respectively. Comparable to findings in patients with psychosis [31], we hypothesized that in 22q11DS, ACC glutamate concentration would be inversely correlated with striatal dopamine D2/3R availability. Additionally, we investigated the association between (1) glutamate, glutamine, and Glx concentrations in the ACC and striatum and (2) ACC volumes in individuals with 22q11DS and healthy volunteers. We hypothesized that higher frontal glutamate, glutamine, and Glx concentrations would be related to lower ACC volumes in patients. The third aim of the present study was to explore the association between cognitive functioning and dopamine D2/3R availability in the ACC and striatum in individuals with 22q11DS.

2. Materials and methods

2.1. Participants

A total of 17 non-psychotic adult individuals with 22q11DS were recruited through the National Adult 22q11DS Outpatient Clinic at Maastricht University Medical Centre and

through the Dutch 22q11DS family network. In addition, 20 age- and sex-matched healthy volunteers were enrolled via social media and advertisement. All participants were recruited as part of a 7T ^{1}H-MRS study.[21] In addition, a subgroup of 22q11DS patients participated in an [^{18}F]fallypride PET study.[32] Recruitment was carried out as previously described.[21,32] Briefly, inclusion criteria were (1) 18–65 years of age and, for adults with 22q11DS, (2) the mental capacity to give informed consent; and (3) a confirmed diagnosis of 22q11DS by fluorescence *in situ* hybridization (FISH), microarray, or multiplex ligation-dependent probe amplification (MLPA). For both groups, exclusion criteria were (1) a history of psychosis as determined by the Mini International Neuropsychiatric Interview (MINI),[33] (2) recreational drug use 4 weeks before participation, (3) previous or current use of stimulant or antipsychotic medication, (4) contraindications for PET and/or magnetic resonance imaging (MRI), and for female participants, (5) pregnancy. Ethical permission was obtained from the Medical Ethical Committee of Maastricht University (The Netherlands; METC142046, NL49834.068.14). Written informed consent was obtained from every participant.

2.2. Procedure and instruments

All subjects underwent ^{1}H-MRS to assess glutamate, glutamine, and Glx concentrations in the right striatum and ACC. Furthermore, a subgroup of ten individuals with 22q11DS underwent [^{18}F]fallypride PET to assess dopamine $D_{2/3}$R availability in the putamen, caudate nucleus (CNC), ventral striatum (VST), and ACC. Cognitive performance was assessed in all subjects with the Cambridge Neuropsychological Test Automated Battery (CANTAB).[34] Seven cognitive domains were assessed with multiple tasks: visual learning and memory, verbal learning and memory, working memory, attention and vigilance, processing speed, reasoning and problem solving, and social cognition (see eTable 1). The FSIQ was determined by the use of the shortened version of the Wechsler Adult Intelligence Scale, version 3 (WAIS-III).[35] The MINI was used to verify the absence of psychiatric disorders.[33] All tests were administered on the same day as the ^{1}H-MRS scan. Additionally, urine drug screening was performed to assure all subjects were free of recreational drugs (i.e., amphetamines, benzodiazepines, cannabis, cocaine, methamphetamines, and opiates). Furthermore, all female participants tested negative for pregnancy in a separate urine screening.

2.3. ^{1}H magnetic resonance spectroscopy and structural MRI

^{1}H-MRS spectra were acquired on a MAGNETOM 7T MR scanner (Siemens Healthineers, Erlangen, Germany) with a stimulated echo acquisition mode (STEAM) sequence (TE = 6.0 ms, TR = 5.0 s, NA = 64, flip angle = 90°).[36] Spectroscopy voxels were manually placed on the right striatum and ACC (Figure 1). LCModel version 6.3-1L[37] was used to analyze the ^{1}H-MRS spectra by use of a GAMMA-simulated basis set[38]

and to estimate concentrations of glutamate, glutamine, and Glx. Metabolite analyses were restricted to spectra with a Cramer–Rao lower bound ≤ 20%. Glutamate, glutamine, and Glx concentrations were corrected for the proportion of CSF as described in Quadrelli et al. (2016).[39] An anatomical weighted image was obtained using a magnetization-prepared two rapid acquisition gradient-echo (MP2RAGE) sequence (TR = 4.5 s, TE = 2.39 ms, TI_1 = 0.90 s, TI_2 = 2.75 s, flip angle$_1$ = 5°, flip angle$_2$ = 3°, voxel size = 0.9 mm isotropic, matrix size = 256 × 256 × 192).[40] ACC volumes were calculated by use of Freesurfer, version 6,[41] as described in Serrarens et al. (2022).[42] A detailed description of the ^{1}H-MRS procedure can be found in Vingerhoets et al. (2020).[21]

2.4. Positron emission tomography

Before the start of the PET scan, a 10 min low-dose 68Ge/68Ga transmission scan was obtained for attenuation correction purposes. Subsequently, approximately 200 MBq [^{18}F]fallypride was administered, followed by 120 min of dynamic PET acquisition, as described in Kasanova et al. (2017).[43] The previously collected T1-weighted image was used for coregistration purposes. SPM2 (Wellcome Trust, UK) was used to realign the [^{18}F]fallypride frames. The PMOD software package (v. 3.6, PMOD Technologies Ltd., Zurich, Switzerland) was used to execute an automatic preprocessing protocol. Realigned PET images were coregistered to the individual T1-weighted image. Afterwards, the individual T1-weighted images were spatially normalized to standard Montreal Neurological Institute (MNI) space in PMOD. PET images were spatially normalized using the same spatial transformation. For each patient, the T1-weighted images were segmented into white matter, grey matter, and cerebrospinal fluid within native MRI space. The PMOD PNEURO tool was used for automatic delineation of the regions of interest (ROIs) by use of the N30R83 Hammers probabilistic atlas.[44] The atlas was adjusted to the T1-weighted scan of the subject. The following ROIs were investigated: (1) ACC, mean, left, and right; (2) putamen; (3) CNC; (4) VST; and (5) cerebellum (i.e., cerebellar hemispheres without the vermis; reference region).[44] Subsequently, the linear extension of the SRTM (LSRRM)[45] was used to estimate kinetic parameters and the time–activity curves (TACs) for all striatal and frontal ROIs. Using an in-house MATLAB (version 6.5) script, [^{18}F]fallypride binding potential (BP_{ND}) was estimated in each ROI.[45] A detailed description of the PET procedure can be found in van Duin et al. (2018)[32] and Kasanova et al.(2017).[43]

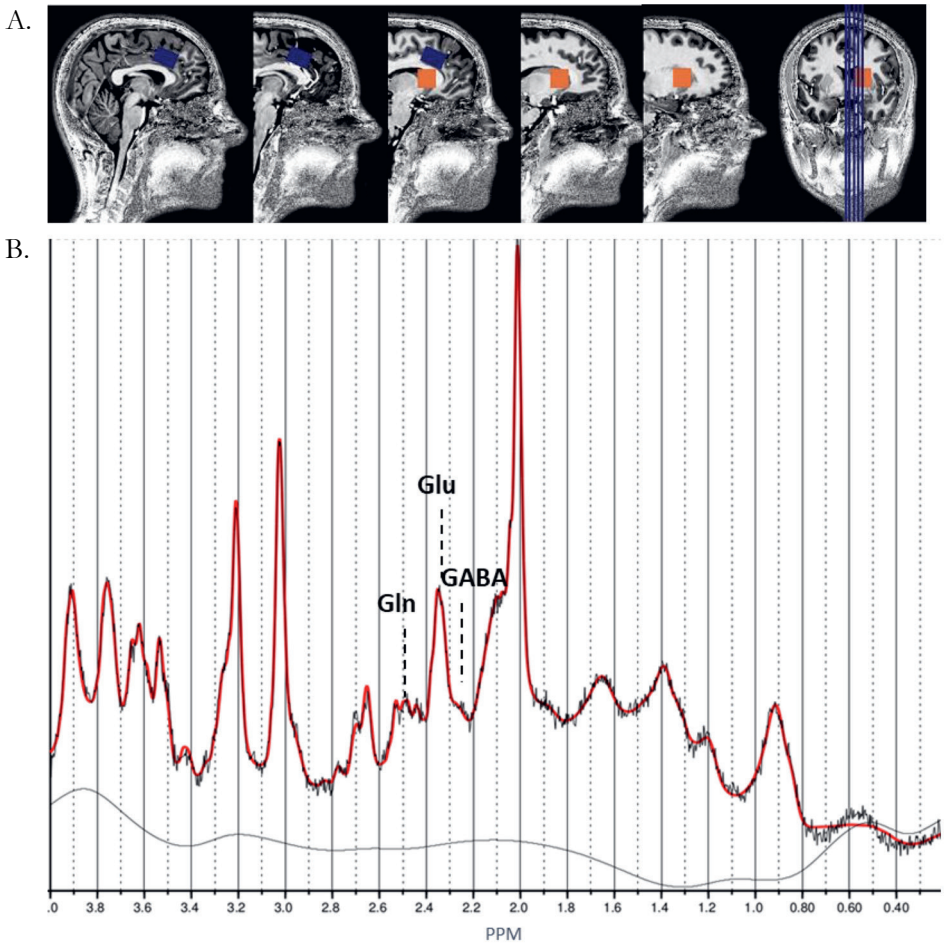

Figure 1. Proton magnetic resonance spectroscopy (^{1}H-MRS) voxel placement and ^{1}H-MRS spectrum. (A) Sagittal and coronal views of MRS voxels displayed on a single subject's T1 structural image. The blue lines in the coronal view (far-right image) indicate the locations of the sagittal views from to right. The orange box indicates the location of the voxel in the striatum. The blue box indicates the location of the voxel in the ACC. (B) Example of an ACC spectrum from a healthy control by LCModel. Reprinted from Vingerhoets et al. (2020).[21]

Abbreviations: ACC, anterior cingulate cortex; GABA, y-aminobutyric acid; Gln, glutamine; Glu, glutamate; PPM, parts per million.

2.5. Statistical analyses

All statistical analyses were performed in IBM SPSS Statistics (version 22). Differences in sample characteristics, including age, sex, selective serotonin reuptake inhibitor (SSRI) use, and FSIQ, were assessed using chi-square, Fisher's exact, or Mann–Whitney U tests. Subsequently, cognitive domain scores were calculated by (1) reverse coding the scales of some outcome measures such that higher scores corresponded to better

performance on all tasks, (2) calculating z-scores and removing outliers (i.e., z-scores lower than -3 or higher than 3), and (3) summing all z-scores within a cognitive domain and dividing by the number of outcome measures within the domain. A composite score was calculated by computing the sum of all seven domain scores. Finally, given the limited sample size and its robustness to the influence of outliers, Spearman's correlation coefficient was used to examine the associations between (1) striatal and frontal dopaminergic and glutamatergic functioning in individuals with 22q11DS, (2) striatal and ACC glutamatergic functioning and ACC volumes in individuals with 22q11DS and healthy controls, and (3) striatal and frontal dopaminergic and cognitive functioning in 22q11DS. Bonferroni correction was used to correct for multiple testing. Consequently, for the first, second, and third objectives, p-values < 0.0083 (0.05/(3 [^{1}H-MRS metabolites] × 2 [^{1}H-MRS brain regions]), < 0.0125 (0.05/4 [ACC volumes]), and < 0.00555 (0.05/9 [7 cognitive domains, composite score, and FSIQ])) were considered significant, respectively.

Table 1. Sample demographics.

	PET 22q11DS (N=10) Mean (SD)	**MRI 22q11DS (N=17)** Mean (SD)	**MRI HC (N=20)** Mean (SD)	**Statistic**	**p-value**
Sex (F/M)	5/5	11/6	12/8	0.09	0.77[a]
Age, years	37.07 (11.12)	34.17 (11.41)	30.70 (8.20)	145.00	0.46[2]
FSIQ	82.60 (12.23)	76.65 (12.32)	120.21 (16.23)[c]	4.50	**<0.001**[b]
Smoking in the previous year (yes/no)	0/9[c]	2/13[c]	2/16[c]	NA	1.00[d]
Current SSRI use (yes/no)	1/9	2/15	1/19	NA	0.58[d]
Time between MRI and PET scan, days	180.10 (349.52)	NA	NA	NA	NA

Abbreviations: F, female; FSIQ, full-scale intelligence quotient; HC, healthy control; M, male; MRI, magnetic resonance imaging; PET, positron emission tomography; SSRI, selective serotonin reuptake inhibitor; 22q11DS, 22q11.2 deletion syndrome. Significant results are bold.

[a]Differences in sample demographics between MRI 22q11DS and MRI HC samples were assessed using a Chi-square test. [b]Differences in sample demographics between MRI 22q11DS and MRI HC samples were assessed using a Mann–Whitney U test. [c]Data on FSIQ was not available for one HC. Data on smoking status was not available for two patients with 22q11DS and two HCs. [d]Differences in sample demographics between MRI 22q11DS and MRI HC samples were assessed using a Fisher's exact test.

3. Results

3.1. Demographics

Demographic details of participants (i.e., 22q11DS and healthy controls that underwent MRI scanning, as well as a subgroup of patients with 22q11DS that also underwent PET

scanning) are shown in Table 1. There were no between-group differences in sex, age, smoking status, and SSRI use between 22q11DS individuals and healthy controls who underwent MRI. However, as expected, patients with 22q11DS had significantly lower FSIQ-scores compared to healthy controls ($U = 4.50$, $p < 0.001$).

3.2. Association between dopaminergic and glutamatergic functioning in 22q11DS

Within the 22q11DS group, glutamate, glutamine, and Glx concentrations in the ACC or striatum were not significantly correlated with mean dopamine $D_{2/3}R$ availability in the ACC, CNC, putamen, or VST (Table 2). In addition, no significant associations were found between glutamate, glutamine, or Glx concentrations in the ACC or striatum and left or right $D_{2/3}R$ availability in the CNC, putamen, or VST (eTable 2).

Table 2. Associations between dopaminergic and glutamatergic functioning in 22q11DS[a].

	ACC glutamate	**ACC glutamine**	**ACC Glx**	**Striatum glutamate**	**Striatum glutamine**	**Striatum Glx**
BP_{ND} [^{18}F]fallypride ACC	r = 0.15 p = 0.68	r = 0.01 p = 0.99	r = 0.07 p = 0.86	r = 0.47 p = 0.17	r = 0.18 p = 0.64	r = 0.56 p = 0.09
BP_{ND} [^{18}F]fallypride CNC (mean)	r = -0.33 p = 0.35	r = -0.27 p = 0.45	r = -0.46 p = 0.19	r = 0.21 p = 0.56	r = 0.27 p = 0.49	r = 0.17 p = 0.65
BP_{ND} [^{18}F]fallypride putamen (mean)	r = -0.52 p = 0.13	r = -0.31 p = 0.39	r = -0.46 p = 0.19	r = -0.10 p = 0.78	r = 0.10 p = 0.80	r = -0.07 p = 0.86
BP_{ND} [^{18}F]fallypride VST (mean)	r = -0.31 p = 0.39	r = -0.20 p = 0.58	r = -0.30 p = 0.41	r = 0.30 p = 0.41	r = -0.33 p = 0.38	r = 0.19 p = 0.60

Abbreviations: ACC, anterior cingulate cortex; BP_{ND}, binding potential; CNC, caudate nucleus; Glx, glutamate plus glutamine; VST, ventral striatum.

[a]Only correlations with $p < 0.0083$ were deemed statistically significant ($0.05/(3$ [^{1}H-MRS metabolites] $\times$ 2 [^{1}H-MRS brain regions]); Bonferroni correction).

3.3. Association between glutamatergic functioning and ACC volumes in 22q11DS and controls

Within the 22q11DS group, no significant correlations were found between left and right rostral and caudal ACC volumes and glutamate, glutamine, or Glx concentrations in the ACC or striatum (Table 3). Furthermore, within the healthy control group, significant positive associations were found between right rostral ACC volume and glutamate concentration in the ACC (effect size measure, $r = 0.49$), left caudal ACC volume, and glutamine concentration in the ACC (effect size measure $r = 0.51$), as well as between right caudal ACC volume and Glx concentration in the ACC (effect size measure $r = -0.53$). However, these associations were no longer significant after Bonferroni correction.

Table 3. Associations between glutamatergic functioning and ACC volumes in 22q11DS and controls[a].

		Left rostral ACC volume	Right rostral ACC volume	Left caudal ACC volume	Right caudal ACC volume
22q11DS	ACC glutamate	r = 0.34 p = 0.19	r = 0.05 p = 0.85	r = 0.36 p = 0.18	r = -0.37 p = 0.16
	ACC glutamine	r = 0.30 p = 0.25	r = -0.01 p = 0.98	r = 0.03 p = 0.92	r = -0.12 p = 0.67
	ACC Glx	r = 0.01 p = 0.98	r = -0.30 p = 0.26	r = 0.14 p = 0.59	r = -0.43 p = 0.09
	Striatum glutamate	r = -0.45 p = 0.08	r = -0.05 p = 0.85	r = 0.09 p = 0.74	r = 0.01 p = 0.96
	Striatum glutamine	r = -0.05 p = 0.85	r = 0.20 p = 0.48	r = 0.12 p = 0.67	r = -0.21 p = 0.44
	Striatum Glx	r = 0.02 p = 0.94	r = 0.12 p = 0.66	r = 0.31 p = 0.25	r = -0.09 p = 0.73
HC	ACC glutamate	r = 0.22 p = 0.36	r = 0.49 **p = 0.03**	r = -0.14 p = 0.54	r = 0.12 p = 0.61
	ACC glutamine	r = 0.09 p = 0.71	r = -0.15 p = 0.54	r = 0.51 **p = 0.03**	r = -0.11 p = 0.65
	ACC Glx	r = 0.10 p = 0.67	r = 0.25 p = 0.29	r = 0.25 p = 0.30	r = -0.53 **p = 0.02**
	Striatum glutamate	r = -0.01 p = 0.97	r = 0.40 p = 0.08	r = -0.18 p = 0.44	r = 0.31 p = 0.18
	Striatum glutamine	r = -0.33 p = 0.23	r = -0.31 p = 0.26	r = -0.07 p = 0.80	r = -0.37 p = 0.18
	Striatum Glx	r = -0.16 p = 0.49	r = 0.14 p = 0.54	r = -0.39 p = 0.09	r = 0.15 p = 0.53

Significant results before Bonferroni correction are bold. *Abbreviations:* ACC, anterior cingulate cortex; Glx, glutamate plus glutamine; HC, healthy control; 22q11DS, 22q11.2 deletion syndrome.
[a]Only correlations with $p < 0.0125$ were deemed statistically significant (0.05/4 [ACC volumes]).

3.4. Association between cognitive functioning and dopamine $D_{2/3}$ receptor availability in 22q11DS

One 22q11DS subject was excluded from the analyses that focused on the cognitive domain attention due to an extreme value. There were no outliers for the other cognitive domains, composite score, or FSIQ. Within the 22q11DS group, mean, left, and right dopamine $D_{2/3}$R availability in the CNC, putamen, and VST were not significantly related to any of the seven cognitive domains, the composite score, or FSIQ (Table 4 and eTable 3), except for dopamine $D_{2/3}$R availability in the left VST and verbal memory (effect size measure, r = -0.70). However, after Bonferroni correction, this association did not remain significant. Furthermore, visual memory, executive functioning, and the composite score were significantly correlated with dopamine $D_{2/3}$R availability in the ACC (although not significant after Bonferroni correction). The results remained the same after correcting for ACC volume (i.e., left, right, caudal, and rostral ACC volumes combined). The association between cognitive and glutamatergic functioning was previously reported in the same sample and is therefore not re-examined in this study.[21]

Table 4. Association between cognitive functioning and dopamine $D_{2/3}$ receptor availability in 22q11DS[a].

	BP_{ND} [^{18}F]fallypride ACC	BP_{ND} [^{18}F]fallypride CNC (mean)	BP_{ND} [^{18}F]fallypride putamen (mean)	BP_{ND} [^{18}F]fallypride VST (mean)
Visual memory	r = -0.72	r = 0.21	r = 0.36	r = -0.21
	p = 0.02	p = 0.56	p = 0.31	p = 0.56
Verbal memory	r = -0.62	r = 0.09	r = -0.08	r = -0.56
	p > 0.05	p = 0.80	p = 0.83	p = 0.09
Working memory	r = -0.63	r = -0.03	r = 0.24	r = -0.26
	p > 0.05	p = 0.93	p = 0.50	p = 0.47
Attention[b]	r = -0.55	r = 0.20	r = 0.07	r = -0.33
	p = 0.13	p = 0.61	p = 0.87	p = 0.38
Processing speed	r = -0.03	r = 0.15	r = -0.13	r = -0.02
	p = 0.93	p = 0.68	p = 0.73	p = 0.96
Executive functioning	r = -0.74	r = -0.29	r = -0.08	r = -0.24
	p = 0.01	p = 0.42	p = 0.83	p = 0.51
Social cognition	r = -0.60	r = 0.09	r = 0.12	r = -0.46
	p = 0.07	p = 0.80	p = 0.74	p = 0.18
Composite score	r = -0.78	r = 0.06	r = 0.07	r = -0.43
	p = 0.01	p = 0.88	p = 0.86	p = 0.21
FSIQ	r = -0.45	r = 0.26	r = 0.34	r = 0.27
	p = 0.19	p = 0.47	p = 0.34	p = 0.46

Significant results before Bonferroni correction are bold.

Abbreviations: ACC, anterior cingulate cortex; BP_{ND}, binding potential; CNC, caudate nucleus; FSIQ, full-scale intelligence quotient; VST, ventral striatum.

[a]Only correlations with $p < 0.00555$ were deemed statistically significant (0.05/9 [seven cognitive domains, composite score, and FSIQ]; Bonferroni correction). [b]One 22q11DS subject was excluded from the analyses that focused on cognitive domain attention due to an extreme value.

4. Discussion

The aims of this study were threefold: (I) to investigate the association between dopaminergic and glutamatergic markers in 22q11DS, (II) to examine the association between glutamatergic functioning and ACC volumes in 22q11DS and healthy controls, and (III) to investigate the association between cognitive functioning and dopamine $D_{2/3}$R availability in 22q11DS. Although we did not find significant associations after Bonferroni correction between any of the abovementioned outcomes, our results provide useful insights. Despite the limited sample size, some associations reached statistical significance with medium-to-large effect sizes.

4.1. Association between dopaminergic and glutamatergic functioning in 22q11DS

We did not find a significant association between dopaminergic and glutamatergic functioning in 22q11DS. This result is not in line with previous findings in patients with psychosis[31] and individuals at ultra-high risk of psychosis.[46] The lack of associations between dopaminergic and glutamatergic markers in our study is likely related to the small sample size, as only ten participants underwent both dopaminergic and

glutamatergic imaging. Another speculative explanation is that the participants in our study did not have pronounced psychotic symptoms, as opposed to the participants in Jauhar et al. (2018)[31] and Stone et al. (2010),[46] which employed [^{18}F]F-DOPA PET to investigate dopamine synthesis capacity. This could suggest that the association between glutamatergic and dopaminergic functioning might be a state characteristic for psychotic symptoms. However, this is speculative and should be examined in future research. Despite the lack of statistical significance, we did report some medium effect sizes comparable to the effect size reported in Jauhar et al. (2018).[31] Therefore, we cannot rule out that a significant association between glutamatergic and dopaminergic markers exists in 22q11DS. Moreover, dopamine $D_{2/3}$R availability in the striatum, as measured with PET or single photon emission computed tomography (SPECT), is determined by multiple aspects: endogenous concentrations of dopamine in the synaptic cleft, affinity of the used radiotracer for the dopamine $D_{2/3}$R, and receptor density.[47,48] Therefore, compensatory mechanisms that cancel each other out may explain the absence of associations between dopamine $D_{2/3}$R availability and ACC glutamate/glutamine/Glx concentrations in 22q11DS. Finally, Jauhar et al. (2018)[31] did not find a significant relation between Glx concentration in the ACC and striatal dopamine synthesis capacity in patients with psychosis, which is in line with our findings. Future studies should be conducted with a larger sample, making use of multimodal imaging techniques to further elaborate these exploratory findings and to advance our understanding in this area.

4.2. Association between glutamatergic functioning and ACC volumes in 22q11DS and controls

Prior to Bonferroni correction, we found an association between right rostral ACC volume and glutamate concentration in the ACC, between left caudal ACC volume and glutamine concentration in the ACC, as well as between right caudal ACC volume and Glx concentration in the ACC in healthy controls. However, no such associations were found in 22q11DS. This suggests that the associations between ACC volumes and glutamate/glutamine/Glx concentrations in the ACC may differ between groups. However, additional research is needed to elucidate this phenomenon. Schizophrenia and 22q11DS are characterized by a loss of brain volume,[19,49] and previous research has suggested that the glutamatergic system might be involved in the mechanism underlying this loss of brain volume.[50,51] The glutamatergic system is of particular interest due to its potential to cause neuroexcitotoxicity, which may lead to reduced grey matter volume. The excitotoxicity hypothesis of schizophrenia proposes that in at least a subgroup of patients with schizophrenia, excitotoxic neuronal cell death occurs in cortical and hippocampal regions via the disinhibition of glutamatergic projections to these regions.[18] Multiple studies have reported associations between glutamatergic and structural measures in patients with psychosis. In unmedicated patients with

schizophrenia but not healthy controls, increased glutamatergic levels in the hippocampus have been associated with reduced hippocampal volume.[52] In addition, Plitman et al. (2016)[53] found a negative association between Glx levels in the precommissural dorsal caudate and precommissural caudate volume in patients with a first non-affective episode of psychosis. This was not the case for healthy controls. Our preliminary results are in line with these findings, suggesting that the association between glutamatergic functioning and brain volume differs between patients with psychosis and controls. Because 22q11DS is associated with an increased risk of developing psychosis,[4] neuroexcitotoxicity due to excessive glutamate might also occur more frequently in at least a subgroup of individuals with 22q11DS who develop psychosis. A previous study did not reveal increased hippocampal glutamate, glutamine, or Glx levels in non-psychotic 22q11DS patients compared to controls but revealed increased hippocampal glutamate and Glx concentrations in 22q11DS patients who developed schizophrenia compared to 22q11DS patients who did not.[22] This suggests that patients who develop psychosis might benefit from drugs that affect the glutamatergic system. Further studies should be conducted to elaborate on this hypothesis.

4.3. Association between cognitive functioning and dopamine $D_{2/3}$ receptor availability in 22q11DS

Within the 22q11DS group, the association between dopamine $D_{2/3}$R availability in the left VST and verbal memory, as well as the associations between dopamine $D_{2/3}$R availability in the ACC and visual memory, executive functioning, and the composite score, reached statistical significance. Again, the effect sizes are noteworthy (i.e., corresponding to strong effects).[54] This suggests that our hypothesis of a correlation between dopamine $D_{2/3}$R availability and cognitive functioning might be verified in a larger sample. Multiple studies have demonstrated a positive association between striatal dopamine $D_{2/3}$R availability and executive function in healthy individuals.[55-59] Our findings suggest an inverse rather than a positive correlation. This discrepancy might be explained by the inverted U-shaped curve model presented in Goldman-Rakic et al. (2000).[60] According to this model, hypo- and hyperstimulation of the dopamine D_1 receptor are associated with deteriorated working memory functioning. The inverted U-shaped curve model might also apply to other aspects of dopaminergic functioning, such as dopamine $D_{2/3}$R availability, as well as to other cognitive domains. Moreover, Cox et al. (2015)[61] reported an inverted U-shaped association between learning from negative feedback and striatal dopamine $D_{2/3}$R availability. Additionally, dopamine $D_{2/3}$R availability might only be associated with specific aspects of cognitive functioning, whereas a previous study in healthy individuals found that D_2 receptor availability in the limbic striatum was related to performance on tests of episodic memory but not to performance on tests of general knowledge or verbal fluency.[56] In

addition, the association between dopamine $D_{2/3}R$ availability and specific aspects of cognitive functioning might be region-specific, as D_2 receptor availability in the associative and sensorimotor subdivisions of the striatum of healthy individuals were found to be less correlated to episodic memory but were instead found to be associated with non-episodic tests.[56] Future studies should further investigate the association between cognitive and dopaminergic measures, as well as the potential of dopaminergic drugs to reduce cognitive deficits in 22q11DS.

4.4. Strengths and limitations

A major strength of this study is the use of multiple imaging modalities (i.e., 7T MRI and PET) in a sample of adults with 22q11DS who were not psychotic and antipsychotic-free at the time of inclusion. However, some limitations have to be taken into account as well. First, as previously mentioned, the sizes of our MRI and PET samples were small due to the difficulty in recruiting this study population; therefore, this study lacked the power to detect significant associations. Secondly, although the majority of the sample did not use psychotropic medication, two patients with 22q11DS and one healthy control used SSRIs. Because SSRIs indirectly inhibit dopaminergic neurotransmission,[62] participants were asked to refrain from this medication on the day of the scanning to limit acute effects on the glutamatergic and dopaminergic systems. Third, we investigated the dopaminergic system during rest and not following pharmacological, behavioral, or cognitive challenges. Therefore, our study does not provide insight into whether other aspects of dopaminergic functioning are altered in 22q11DS. Fourth, the phenotypic expression of 22q11DS is highly heterogeneous and includes congenital heart disease.[3] Consequently, many patients with 22q11DS carry medical implants and were therefore not allowed to participate in the 7T ^{1}H-MRS study. In addition, because the majority of 22q11.2DS patients with psychosis use antipsychotic medication and are often not mentally competent to provide informed consent, we did not include these patients in the current study to minimize heterogeneity in the sample. This may have caused a selection bias of relatively healthy patients and made it difficult to generalize the results to the whole 22q11DS population. Finally, although, contrary to 3T, 7T MRI glutamate and glutamine can be reliably distinguished, it does not enable detailed localization of glutamatergic metabolites (e.g., pre- versus postsynaptic and intracellular versus extracellular).

4.5. Implications and suggestions for future work

Although we did not find significant associations after Bonferroni correction between dopaminergic, glutamatergic, and cognitive functioning, some associations reached statistical significance. Our findings suggest that the association between ACC volumes and glutamate, glutamine, and Glx concentrations in the ACC are likely differ between

individuals with 22q11DS compared to healthy controls. In addition, dopamine $D_{2/3}R$ availability seems to be related to cognitive functioning, although the causal relationships between cognitive domains and dopaminergic functioning are yet unknown. Future research with larger samples is needed to further elucidate both of these hypotheses. Furthermore, ACC glutamatergic functioning might not be related to dopamine $D_{2/3}R$ availability in 22q11DS but instead be associated with other aspects of dopaminergic functioning, such as striatal dopamine synthesis capacity or dopamine transporter expression. To investigate this hypothesis, additional studies required that make use of other PET and/or SPECT radiotracers (i.e., [^{18}F]F-DOPA, [^{11}C]DTBZ, or [^{123}I]FP-CIT) combined with ^{1}H-MRS imaging.

5. Conclusions

This exploratory study addresses the relationships between dopaminergic, glutamatergic, and cognitive functioning in individuals with 22q11DS using ^{1}H-MRS and [^{18}F]fallypride PET. Although our results did not reach statistical significance, the effect sizes warrant future research on this topic. Additional studies with larger samples are needed to further elucidate our findings.

6. Acknowledgements

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7. Author contributions

Conceptualization: TvA; Methodology: TvA, JB; Formal analysis: CvH, CV, DT. JC, JR; Investigation: CV, DT; Writing - original draft preparation: CvH, CV; Writing - review and editing: CvH, DT. JR, JC, JB, TvA, CV. All authors have read and agreed to the published version of the manuscript.

8. Declarations of interest

The authors declare no conflict of interest.

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Appendix to Chapter

Supplementary information can be found in the eBook (pages 125-127):

Part

Neurobiology of individuals with a non-affective psychotic disorder

Chapter

The substantia nigra in the pathology of schizophrenia: a review on post-mortem and molecular imaging findings

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Abstract

Dysregulation of striatal dopamine is considered to be an important driver of pathophysiological processes in schizophrenia. Despite being one of the main origins of dopaminergic input to the striatum, the (dys)functioning of the substantia nigra (SN) has been relatively understudied in schizophrenia. Hence, this paper aims to review different molecular aspects of nigral functioning in patients with schizophrenia compared to healthy controls by integrating post-mortem and molecular imaging studies. We found evidence for hyperdopaminergic functioning in the SN of patients with schizophrenia (i.e., increased AADC activity in antipsychotic-free/-naïve patients and elevated neuromelanin accumulation). Reduced GABAergic inhibition (i.e., decreased density of GABAergic synapses, lower VGAT mRNA levels and lower mRNA levels for $GABA_A$ receptor subunits), excessive glutamatergic excitation (i.e., increased NR1 and Glur5 mRNA levels and a reduced number of astrocytes), and several other disturbances implicating the SN (i.e., immune functioning and copper concentrations) could potentially underlie this nigral hyperactivity and associated striatal hyperdopaminergic functioning in schizophrenia. These results highlight the importance of the SN in schizophrenia pathology and suggest that some aspects of molecular functioning in the SN could potentially be used as treatment targets or biomarkers.

1. Introduction

Schizophrenia is a severe mental disorder characterized by positive symptoms including hallucinations and delusions, negative symptoms such as social withdrawal and avolition, and cognitive symptoms including deficits in executive functioning and working memory.[1] A complex pathology is thought to underlie schizophrenia. The dopamine hypothesis proposes a framework that links the interaction between multiple risk factors (e.g., drug use, genes, and stress) and frontotemporal dysfunction to a final common pathway of dopamine dysregulation, more specifically striatal hyperdopaminergia. Striatal hyperdopaminergia is thought to alter the appraisal of stimuli, subsequently leading to the development of psychotic symptoms. Converging evidence showed that the striatal hyperdopaminergia is primarily located presynaptically (for descriptions of versions I, II, and III of the dopamine hypothesis see Snyder (1976)[2]; Davis et al. (1991)[3]; and Howes & Kapur (2009),[4] respectively). Dopaminergic neurons primarily originate from two midbrain structures: the substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA). Projections from the SNc to the dorsal striatum form the nigrostriatal dopaminergic pathway, while projections from the VTA to the nucleus accumbens/ventromedial striatum form the mesolimbic pathway and from the VTA to cortical regions (in particular the frontal cortex) the mesocortical pathway. The mesocortical pathway and frontal hypodopaminergia have been implicated in the cognitive symptoms of schizophrenia.[3] Originally, dysfunction of the mesolimbic pathway was thought to underlie the striatal hyperdopaminergia and the psychotic symptoms, but insights from neuroimaging studies suggest that dopaminergic dysfunction in schizophrenia is greatest within the nigrostriatal pathway (as reviewed by McCutcheon et al. (2019)[5]). Several studies have identified the associative striatum as the main region of increased striatal dopaminergic functioning in psychosis.[6-8] The associative striatum receives dopaminergic innervation from primarily the ventral tier of the SNc[9] and it has been hypothesized that the increase in striatal dopamine functioning might be related to upstream alterations in the substantia nigra (SN). Despite the importance of dopamine dysregulation in the pathology of schizophrenia, and the SN as the main origin of dopaminergic neurons of the nigrostriatal pathway, this midbrain structure has been relatively understudied in patients with schizophrenia.

Dopaminergic abnormalities on their own do not explain all facets of schizophrenia pathology. Other neurotransmitters, such as glutamate and γ-aminobutyric acid (GABA) are likely to be involved. This suggestion is based on the observation that blocking of N-methyl-D-aspartate (NMDA) receptors on GABAergic interneurons in the cortex by antagonists, such as phencyclidine (PCP) and ketamine, results in schizophrenia-like symptoms in healthy individuals and worsens these symptoms in patients.[10,11] Alterations in GABAergic and glutamatergic functioning have been widely studied in schizophrenia (as reviewed by Egerton et al. (2017,2020)[12,13]) and

the glutamate hypothesis suggests hypo-functioning of NMDA receptors on GABAergic interneurons in the cortex, which leads to excessive glutamate release.[14] The dopamine and glutamate hypotheses are not mutually exclusive. In fact, the glutamate hypothesis can function as an extension of the dopamine hypothesis, and combined they propose that presynaptic striatal hyperdopaminergia in patients with schizophrenia might be secondary to alterations in glutamatergic functioning.[15] Most of the studies on GABAergic and glutamatergic functioning in schizophrenia did not investigate the SN, even though the SN pars reticulata (SNr) is mainly involved in GABAergic signalling.[16] This suggests that nigral glutamatergic and GABAergic functioning might also be relevant for schizophrenia pathology.

Hence, we aim to review the molecular alterations that occur in the SN of patients with schizophrenia, investigate how these changes may contribute to schizophrenia pathology and identify knowledge gaps. We investigated these aims by reviewing post-mortem and molecular imaging studies (i.e., by use of positron emission tomography [PET], single photon emission computed tomography [SPECT], proton magnetic resonance spectroscopy [^{1}H-MRS], or neuromelanin-sensitive magnetic resonance imaging [NM-MRI]) that investigated different molecular aspects of nigral functioning in patients with schizophrenia compared to controls. We first focus on the dopaminergic signalling pathway within the SN. Next, we discuss the nigral glutamatergic and GABAergic signalling pathways. We then overview other molecular alterations in the SN that might be relevant for schizophrenia pathology. Finally, we integrate the different topics, place our findings in the context of what has been found by animal studies, and identify avenues for future research.

2. Dopaminergic alterations in the substantia nigra of patients with schizophrenia

Within dopaminergic synapses, tyrosine hydroxylase (TH) converts tyrosine into l-3,4-dihydroxyphenylalanine (L-DOPA) and is the rate-limiting enzyme for dopamine production (Figure 1).[17] Multiple post-mortem studies reported elevated TH protein levels in the SN[18-20] and increased TH messenger RNA (mRNA) levels in the SNc[21] of patients with schizophrenia compared to controls (Table 1). However, other studies found no differences in TH protein levels in the SN[22] or TH mRNA levels in the SN[22,23] or midbrain (which includes the SN and ventral tegmental area [VTA]).[24] In addition, decreased TH protein levels in the SN/VTA[24] and lower TH mRNA levels in the SN of patients with schizophrenia relative to controls have also been reported[25] (same cohort as Purves-Tyson et al. (2017),[22] but using a more sensitive quantitative polymerase chain reaction [qPCR] platform). The opposing study outcomes might be explained by differences in exposure to antipsychotic medication, illness duration, cohort size, and sampling area (i.e., SN versus SN/VTA). Rodent studies, however,

suggest that antipsychotic medication does either not change[24] or reduces TH levels.[26] Additionally, in the largest cohort of ~27 patients, Purves-Tyson et al. (2017)[22] found no correlation between (1) measures of antipsychotic drug treatment or illness duration and (2) TH mRNA or protein levels in the SN, supporting that these factors did not change the molecular parameters. TH activity might be differently regulated in different subregions of the midbrain, as Perez-Costas et al. (2012)[24] found decreased and unaltered TH protein levels in the rostro-caudal and mid-caudal parts of the SN/VTA in patients with schizophrenia compared to controls, respectively. Even though these findings underline the importance of regional differences in TH activity in the midbrain, this analysis only included eight patients and six controls. The current data does, therefore, not support a clear increase or decrease of TH protein- and mRNA levels in the SN and underlines the need for larger well-powered studies that consider regional differences within the SN/VTA.

After the conversion of tyrosine to L-DOPA, L-DOPA is subsequently converted into dopamine by aromatic L-amino acid decarboxylase (AADC). *Ex vivo* post-mortem studies have found unaltered AADC mRNA levels in the SN of patients with schizophrenia,[22,23] although there was a trend for lower AADC mRNA levels in patients in one of the studies.[22] Additionally, two *in vivo* [^{18}F]F-DOPA PET imaging studies reported no significant differences in [^{18}F]F-DOPA uptake in the SN[27] or SN/VTA[28] between patients with schizophrenia and healthy controls (HC). The study of Elkashef et al. (2000)[28] may have been less sensitive to detect group differences due to the lower scanner resolution of earlier generation PET scanners. In contrast, two other studies showed elevated [^{18}F]F-DOPA uptake in the SN[18] and midbrain of patients with schizophrenia.[29] These inconsistencies might be explained by differences in antipsychotic medication usage. Although five weeks of antipsychotic treatment did not alter nigral [^{18}F]F-DOPA uptake in the study of Jauhar et al. (2019),[30] a decrease of [^{18}F]F-DOPA uptake in the caudate nucleus, putamen, thalamus, and cortex following at least twenty days of treatment with haloperidol has been reported.[31] Furthermore, Howes et al. (2013)[18] performed a post-hoc analysis and found an increase in nigral [^{18}F]F-DOPA uptake in antipsychotic- free patients compared to HC. No differences were found for antipsychotic-treated patients, suggesting a medication effect that downregulates AADC levels, and consequently [^{18}F]F-DOPA uptake. Additionally, Allen et al. (2012)[32] found a significant increase of [^{18}F]F-DOPA uptake in the SN/VTA of antipsychotic-naïve or -free people at ultra-high risk (UHR) for psychosis who subsequently made the transition to psychosis relative to UHR individuals who did not. Furthermore, a trend was found for an elevation in the SN/VTA [^{18}F]F-DOPA uptake in antipsychotic-naïve transitioned UHR subjects compared to HC. Taking the *ex vivo* and *in vivo* data together, there seems to be increased AADC activity in the SN in

schizophrenia, but only in antipsychotic-free or -naïve patients, whereas antipsychotics seem to reduce AADC activity.

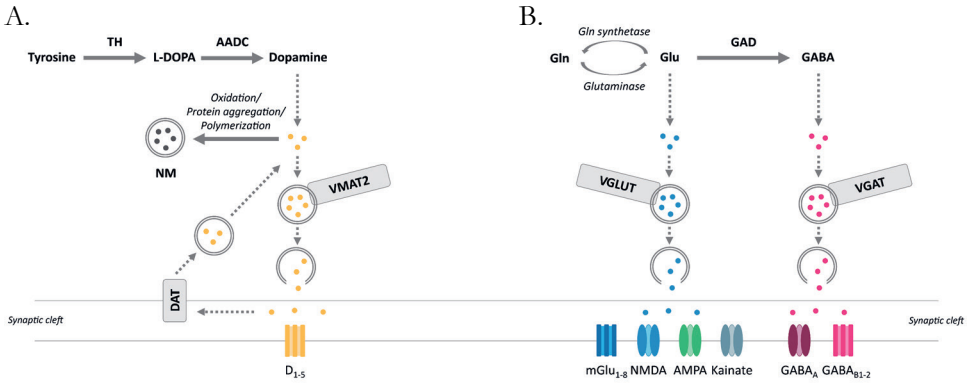

Figure 1. Systematic overview of the dopaminergic, glutamatergic and GABAergic signalling pathways. (A) Schematic overview of the dopaminergic signalling pathway: TH converts tyrosine into L-DOPA, which is then converted into dopamine by AADC. VMAT-2 transports and stores dopamine in synaptic vesicles before dopamine is released into the synaptic cleft. Excess cytosolic dopamine is packaged as NM complexes inside autophagic organelles after a process of iron-dependent oxidation, protein aggregation, and polymerization. Exocytosis of the synaptic vesicles containing dopamine induces dopamine release into the synaptic cleft. After dopamine release, dopamine binds to dopamine metabotropic receptors ($D_{1\text{-}5}$). The presynaptic DAT is responsible for the reuptake of dopamine from the synaptic cleft into the presynaptic terminal. (B) Schematic overview of the glutamatergic/GABAergic signalling pathway: Gln is converted to Glu and Glu to Gln by Glutaminase and Gln synthetase, respectively. GAD synthesizes GABA from Glu. The VGLUT and VGAT transport and store Glu and GABA, respectively, in synaptic vesicles before release in the synaptic cleft. After release, Glu binds to ionotropic receptors (NMDA, AMPA, and kainate) and metabotropic receptors ($mGlu_{1\text{-}8}$). GABA binds to the ionotropic receptor ($GABA_A$) and metabotropic receptor ($GABA_B$).

Abbreviations: AADC, aromatic acid decarboxylase; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; DAT, dopamine transporter; $D_{1\text{-}5}$, dopaminergic (metabotropic) receptors; GABA, γ-aminobutyric acid; $GABA_A$, ionotropic GABA receptor; $GABA_B$, metabotropic receptor; GAD, glutamic acid decarboxylase; Gln, glutamine; Glu, glutamate; kainate, kainate receptor, L-DOPA, l-3,4-dihydroxyphenylalanine; $mGlu_{1\text{-}8}$, metabotropic glutamate receptors; NM, neuromelanin; NMDA, N-methyl-D-aspartate receptor; TH, tyrosine hydroxylase; VGAT, vesicular GABA transporter; VGLUT, vesicular Glu transporter; VMAT-2, vesicular monoamine transporter 2.

After dopamine synthesis, the vesicular monoamine transporter 2 (VMAT-2) is responsible for transporting and storing dopamine (and other monoamines) from the cytoplasm into secretory vesicles (as reviewed by Henry & Scherman (1989[33]). One post-mortem study found a significant decrease in VMAT-2 mRNA levels in the SN of patients with schizophrenia compared to controls.[22] This might suggest the presence of a compensatory mechanism to reduce dopaminergic signal transduction (i.e., less dopamine is stored and subsequently released). However, VMAT-2 binding, as assessed by (+)-α-[^{11}C]DTBZ PET, was elevated in the ventral brainstem (which includes the

SN/VTA and the raphe nuclei) of patients with schizophrenia in comparison with HC.[34] Importantly, these findings should be taken with caution due to the relatively poor resolution of the PET camera and the possibility that ventral (+)-α-[^{11}C]DTBZ uptake in the brainstem predominantly reflects serotonergic instead of dopaminergic projections. Despite the observation that nigral VMAT-2 mRNA levels were not correlated with antipsychotic medication usage or illness duration,[22] additional studies are required to understand potential changes in VMAT-2 in the SN in schizophrenia.

Excess cytosolic dopamine, which is not accumulated into synaptic vesicles, subsequently gets packaged as neuromelanin complexes inside autophagic organelles after a process of iron-dependent oxidation, protein aggregation, and polymerization .[35] Therefore, neuromelanin is thought to be an indirect marker of dopamine synthesis.[36] *In vivo* studies reported both significant elevations[37,38] and no alterations in NM-MRI signal in the SNc of patients with schizophrenia relative to age-matched HC.[36,39,40] The findings of increased NM-MRI signal in the SN of patients are in line with the finding of reduced nigral VMAT-2 mRNA levels.[22] A decrease in nigral VMAT-2 gene expression might contribute to lower levels of VMAT-2 protein and this might result in less efficient vesicular packing of dopamine. This would cause more build-up of dopamine in the cytosol and consequently more formation of neuromelanin complexes. It is noteworthy that Watanabe et al. (2014)[38] included roughly twenty more patients and controls (N = 52 patients, N = 52 controls) than the other studies (Table 1). Therefore, limited sample sizes in the other studies might have hampered finding group differences in nigral NM-MRI signal. Furthermore, illness severity might contribute to the findings, as neuromelanin levels in the SN have been found to correlate positively with psychotic symptom severity and are significantly greater in patients with high psychosis severity (positive subscale scores > 19 on the positive and negative syndrome scale [PANSS]).[39] Alternatively, chronic exposure to antipsychotic treatment might decrease dopaminergic signalling, which could correct for differences in neuromelanin. In addition, as neuromelanin in the SN is known to accumulate during ageing,[41] differences in NM-MRI signal in the SN between older patients and controls might be masked by the age-related accumulation of neuromelanin. Taken together, preliminary evidence suggests increased neuromelanin accumulation in the SN of patients with schizophrenia, although the effect of age, antipsychotic medication, and illness duration and severity should be re-examined in large longitudinal studies.

Following vesicular packaging, dopamine release can be induced by an action potential that causes exocytosis of synaptic vesicles.[42] In the SN, Tseng et al. (2018)[43] reported a significant increase in psychosocial stress-induced dopamine release in antipsychotic-naïve patients with schizophrenia (n = 9) compared to HC and a positive correlation between psychosocial stress-induced [^{11}C]-(+)-PHNO displacement in the SN and whole striatum across all subjects. In contrast, Slifstein et al. (2015)[44] reported

a trend towards lower amphetamine-induced dopamine release capacity in the midbrain (SN/VTA) of antipsychotic-naïve (n = 6) and antipsychotic-free (n = 14) patients with schizophrenia relative to HC using [^{11}C]-FLB457 PET. A possible explanation for this inconsistency is the use of different PET tracers. Whereas [^{11}C]-FLB457 is a very high-affinity antagonist tracer for the dopamine D_2 receptor, [^{11}C]-(+)- PHNO is a dopamine D_3 receptor preferring PET agonist tracer. Agonist tracers appear to be more sensitive to detecting dopamine release compared to antagonist tracers.[45] Furthermore, psychosocial and psychostimulant challenges are proposed to affect endogenous dopamine release differently due to distinctive activation pathways.[43] Stress induces dopamine release endogenously by upregulating cell firing, whereas amphetamine elicits dopamine release pharmacologically by interfering with dopamine signaling.[46] In addition, previous exposure to antipsychotic medication might have downregulated dopamine release capacity in the second study, as antipsychotic medication does not only block postsynaptic striatal dopamine $D_{2/3}$ receptors, but also presynaptic $D_{2/3}$ autoreceptors in the midbrain, which are known to be involved in regulating dopamine release.[47] mRNA levels of the dopamine D_2 receptor splice variant DRD2S are decreased in the SN of post-mortem schizophrenia compared to controls[22] and the D2S splice variant plays a role in presynaptic autoreceptor functioning. This might suggest that there is reduced local autoinhibition via $D_{2/3}$ autoreceptors, and thus less inhibition of somatodendritic dopamine release (i.e., increased nigral dopamine release). Overall, the small number of studies in combination with small sample sizes limits the interpretation of the data and provides no definitive evidence yet for increased or decreased dopamine release in the SN.

After exocytosis, the presynaptic dopamine transporter (DAT) is responsible for the reuptake of dopamine from the synaptic cleft into the presynaptic terminal.[48] Post-mortem studies of patients with chronic schizophrenia reported a significant decrease in DAT mRNA in the SN compared to controls[22,25] (same cohort but the recent study used a more sensitive qPCR platform), but no change in DAT protein levels.[22] The lack of alteration in DAT protein levels may suggest that alterations in DAT mRNA do not influence protein levels in the midbrain or that protein functioning may be changed causing DAT transcription to be altered via feedback mechanisms. Otherwise, DAT utilisation and breakdown may be decreased or translation of DAT protein may be increased, both resulting in no change in DAT protein levels and a decrease in DAT mRNA.[22] Two *in vivo* [^{11}C]PE2I PET studies reported greater DAT binding in the SN/VTA of patients with schizophrenia compared to HC,[49] as well as, no significant differences in the SN.[50] The aforementioned studies included mostly antipsychotic-treated patients. However, it has been shown that antipsychotic medication does not affect DAT binding.[49,51,52] Furthermore, DAT binding seems not to be correlated with the duration of illness or age of onset.[49] Taken together, these

studies suggest that DAT protein levels in the SN are unaltered or may be increased. Although no alterations in DAT binding have been reported in the striatum of patients with schizophrenia,[53] increased DAT functioning in the SN could theoretically serve as a compensatory mechanism to reduce hyperactive functioning of the dopaminergic system in schizophrenia by reducing extracellular dopamine levels. Further studies are required to investigate this hypothesis and understand potential changes in nigral DAT functioning in schizophrenia.

Dopamine that is released into the synaptic cleft can bind to dopamine receptors. In the SN, dopamine receptors primarily belong to the dopamine D_2 and D_3 subtypes.[54,55] The nigral D_2 receptors are mainly functioning as inhibitory autoreceptors that regulate the release of dopamine.[56,57] Purves-Tyson et al. (2017)[22] reported significantly lower mRNA levels of one dopamine D_2 receptor splice variant (i.e., DRD2S) in the SN of post-mortem schizophrenia compared to controls, while other splice variants displayed a trend towards reduced expression (i.e., DRD2L and DRD2Longer). mRNA levels of dopamine D_3 receptor splice variants (i.e., DRD3 full-length and DRD3 non-functional) remained unaltered. Different splice variants have been associated with different functions, with D2S playing a role in presynaptic autoreceptor functioning and D2L mainly acting at postsynaptic sites.[58,59] Another post-mortem study showed an increased [^{3}H]spiperone binding, which is a measure of dopamine D_2 receptor availability, in the SN of neuroleptic-free and -treated patients compared to controls.[60] *In vivo* [^{11}C]-(+)-PHNO PET, [^{18}F]fallypride PET, and [^{123}I]-epidepride SPECT studies report increased,[61] decreased,[62,63] and unaltered[43,64-66] dopamine $D_{2/3}$ receptor availabilities in the SN or midbrain of patients with schizophrenia relative to HC. A meta-analysis that combined most of these studies reported no change in dopamine $D_{2/3}$ receptor availability in the SN of patients with schizophrenia.[67] This is in line with the results of a meta-analysis that reported no significant differences in $D_{2/3}$ receptor availability in the striatum between HC and patients with schizophrenia.[53] Although the vast majority of studies investigated antipsychotic-naïve and -free patients,[61,63-66] some studies did include patients that were treated with antipsychotic medication during the measurements or when passing away.[22,60,62] As antipsychotic medication binds to dopamine $D_{2/3}$ receptors, PET and SPECT tracers compete with antipsychotic medication, as well as, endogenous dopamine for binding to the dopamine $D_{2/3}$ receptors and therefore antipsychotic medication potentially affects the results. However, no differences in nigral dopamine $D_{2/3}$ receptor availabilities have been found between antipsychotic-naïve and - free,[65] or antipsychotic-free and -treated patients.[60] Furthermore, Purves-Tyson et al. (2017)[22] reported no significant correlations between mRNA levels of different dopamine receptors and antipsychotic use. In sum, the results of individual studies do not perfectly align (potentially also due to small sample sizes). Meta-analytic evidence, however,

suggests no alterations in the availability of nigral dopamine $D_{2/3}$ receptors. Unfortunately, PET and SPECT imaging cannot distinguish between D_2 and D_3 receptor binding[68] and, therefore, *in vivo* alterations of specific receptor types cannot be excluded. This remains a topic for future research.

Finally, cytoarchitecture describes the density, morphology, and distribution of cells of the central nervous system.[69] Changes in cytoarchitecture of dopaminergic cells in the SN could, therefore, theoretically influence the functioning of the nigrostriatal pathway. One post-mortem study reported significant increases in nuclear length, nucleolar volume, and nuclear area of nigral dopaminergic neurons in schizophrenia.[70] However, no alterations were found with regard to the somal cross-sectional area. Another study did not find significant alterations in the total number of dopaminergic neurons, total neurons, or their ratio in the SN/VTA of patients with schizophrenia compared to controls.[71] Knowledge regarding nigral neuroarchitecture in schizophrenia and how alterations affect pathology is limited and could be of interest to future research.

3. Glutamatergic alterations in the substantia nigra of patients with schizophrenia

Yamaguchi et al. (2013)[72] found expression of vesicular glutamate transporter (VGLUT) 2 mRNA in the SNc of rats and therefore suggested that there are neurons within the SNc that can participate in glutamatergic neurotransmission. Another animal study showed that there are glutamatergic afferents to neurons in the SNc, which mainly originate in the pedunculopontine and subthalamic nuclei.[73] This glutamatergic input may affect the nigrostriatal pathway by excitatory effects on the dopaminergic neurons and thus could potentially play a role in schizophrenia pathology.

A post-mortem study found that the density of glutamatergic synapses in the central area of the SN, i.e., the area with dopaminergic projections to the associative striatum, was not significantly altered in antipsychotic-treated patients with schizophrenia compared to controls.[74] Within the glutamatergic synapse, VGLUT1-3 store glutamate from the cytoplasm into synaptic vesicles.[75] Two post-mortem studies found no differences in nigral VGLUT1 and VGLUT2 levels between patients with schizophrenia and controls.[19,74] Likewise, VGLUT1 and VGLUT2 levels were similar in the medial, central, and lateral parts of the SN in patients with schizophrenia compared to controls.[74] Importantly, the sample sizes of both studies were small and most patients were not antipsychotic-naïve. As antipsychotic medication reduces the concentration of striatal glutamate,[76] this might have prevented the researchers from finding group differences. In addition, Schoonover et al. (2017)[19] found higher VGLUT2 levels in the SN of antipsychotic-free patients compared to controls, whereas no significant differences were reported between antipsychotic-treated and -free

patients. Interestingly, Mabry et al. (2019)[74] found VGLUT1 levels to be significantly negatively correlated to glutamic acid decarboxylase (GAD) 67 levels in the central area of the SN of controls, whereas this correlation was positive in patients with schizophrenia. Similar patterns were found in the medial and lateral regions of the SN. As GAD67 synthesizes GABA from glutamate, this finding suggests a deviation in the modulation of glutamate concentrations by GAD67 in schizophrenia. In sum, VGLUT1 levels seem not to be altered in patients with schizophrenia, although VGLUT2 levels might be increased in antipsychotic-free patients.

After the release of glutamate into the synaptic cleft, glutamate can bind to ionotropic NMDA, kainate, and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, as well as metabotropic glutamate receptors ($mGlu_{1-8}$).[77] A post-mortem study investigated the expression of NMDA (i.e., NR1, NR2A-D, and NR3A), AMPA (i.e., GluR1-4), and kainate receptor subunits (i.e., GluR5-7 and KA1-2) in the SNc of patients with schizophrenia and controls [21]. Only NR1 and GluR5 mRNA levels were significantly increased in the SN of antipsychotic-treated and -free patients. Furthermore, the expression of several NMDA receptor-associated proteins (i.e., PSD-93, PSD-95, NF-L, SAP102, and Yotiao) in the SNc did not differ between both groups. Altogether, increased nigral NR1 mRNA levels in patients with schizophrenia might lead to the upregulation of NR1 subunits available for the formation of functional NMDA receptors.[21] This could subsequently result in elevated expression of NMDA receptors since NR1 is the obligatory subunit to form NMDA receptors.[78] Even though hypofunctioning of NMDA receptors in the cortex is widely implicated in the glutamate hypothesis of schizophrenia,[12] future research needs to examine whether elevated nigral NR1 mRNA expression could result in increased expression of the NMDA receptor in the SN. In addition to NR1 abnormalities, Mueller et al. (2004)[21] reported nigral GluR5 alterations in schizophrenia. A modification in GluR5 expression may change kainate receptor functioning, as GluR5 homomers and GluR5/6 containing receptors both desensitize faster and recover slower from desensitization than homomeric GluR6 receptors.[79] So far, only one study, with a limited sample size, indicates increased NR1 and GluR5 mRNA levels in the SNc of patients with schizophrenia. No evidence exists for dysregulation of other glutamate receptors in the SN in schizophrenia. To our knowledge, no PET studies on glutamate receptors have been performed although radiotracers for mGluR5 (e.g., [^{18}F]FPEB) and mGluR1 (e.g., [^{18}F]FIMX) have been developed. It would be of interest to confirm the post-mortem findings *in vivo*.

In vivo studies using proton magnetic resonance spectroscopy (^{1}H-MRS) have been conducted to investigate concentrations in the brain of glutamate, glutamine, and their combination Glx (as it is difficult to distinguish the signal of glutamate from glutamine at 3T or lower magnetic field strengths). ^{1}H-MRS is a non-invasive neuroimaging technique that can be used to measure the concentrations of chemical

components within tissues. Often the metabolite concentrations are reported as a ratio to creatine (Cr; a marker of energy metabolism) or N-acetyl-aspartate (NAA; a marker of neuronal integrity). Reid et al. (2013)[80] reported no significant alterations in Glx/Cr levels in the SN of antipsychotic-treated patients with schizophrenia or schizoaffective disorder compared to HC, although they found a trend towards increased Glx/NAA in the SN of patients ($p = 0.05$). An older post-mortem study by Toru et al. (1988)[20] reported no significant changes in nigral glutamate levels between patients with schizophrenia and controls. Glutamate levels in the SN are therefore most likely unaltered in schizophrenia, or possibly slightly increased. For a more definitive answer, future research should preferably investigate the glutamatergic system with high magnetic field (7T instead of 3T) MRI, use absolute quantification instead of a reference metabolite, and assess the potential effect of antipsychotics on glutamate levels. Overall, although most aspects of glutamatergic functioning seem unaltered in the SN of patients, other aspects support increased glutamatergic neurotransmission (i.e., increased NR1 and Glur5 mRNA levels, possibly increased VGLUT2 levels in antipsychotic-free patients, and possibly slightly increased Glx levels). Since glutamate is an excitatory neurotransmitter, an increase in glutamatergic transmission on the dopaminergic neurons in the SN may increase the excitation of the nigrostriatal pathway and may contribute to nigrostriatal hyperdopaminergia.

4. GABAergic alterations in the substantia nigra of patients with schizophrenia

The large majority of inputs to dopaminergic neurons in the SNc are inhibitory GABAergic afferents, which project from the globus pallidus, neostriatum, and SNr.[81] The inhibitory neurotransmitter GABA is therefore an important regulator of the nigrostriatal dopaminergic pathway.

One study investigated synaptic density and reported a significant decrease in the density of GABAergic synapses in the central area of the SN (projecting to the associative striatum) in antipsychotic-treated patients with schizophrenia compared to controls.[74] This may be in line with Feinberg's excessive synaptic pruning hypothesis, which postulates that a critical step in the development of schizophrenia is an incorrectly programmed synaptic pruning process.[82]

Most studies that investigated nigral GABAergic functioning have focused on measuring GAD, which synthesizes GABA from glutamate.[83] The first post-mortem study in antipsychotic-treated patients with schizophrenia found no alterations in nigral GAD levels compared to controls.[84] Another post-mortem study reported a moderate, albeit not significant increase in GAD67 levels, an isoform of GAD, in the SN of antipsychotic-treated patients with schizophrenia compared to controls.[74] In addition, a sub-analysis in which the SN was subdivided into medial, central, and lateral sections

also revealed no significant group differences.[74] These findings are in concordance with the study by Toru et al. (1988),[20] which reported no changes in nigral GABA protein levels between patients with schizophrenia and controls. In contrast, Schoonover et al. (2017)[19] did report a significant increase in GAD67 protein levels in the caudal SN of antipsychotic-treated patients with schizophrenia compared to controls. This was not demonstrated for antipsychotic-free patients. Subsequently, it was proposed that GAD67 levels of antipsychotic-treated patients may be increased due to medication usage, potentially as a compensatory response to inhibit dopaminergic functioning.[74] This is in line with an animal study that showed that the antipsychotic drug olanzapine, but not haloperidol or sertindole, increased GAD67 mRNA in the SNr of rats.[85] Furthermore, chronic treatment with haloperidol, but not olanzapine or sertindole, resulted in increased $GABA_A$ receptor binding in the SNr of rats.[85] A more recently published study, however, reported reduced GAD mRNA and protein levels in the SN of antipsychotic-treated patients with schizophrenia compared to controls.[25] These findings suggests that although antipsychotic treatment might increase GAD levels, this may not be the case for all antipsychotics. As GAD protein levels were found to positively correlate with illness duration in patients with schizophrenia,[25] longer illness durations may further increase GAD67 levels in patients,[25] although this might also be related to longer antipsychotic exposure.

Within the GABAergic neurons, the vesicular GABA transporter (VGAT) is responsible for the vesicular storage and exocytosis of GABA.[86] Purves-Tyson et al. (2021)[25] reported significantly decreased VGAT mRNA in the SN of patients with schizophrenia compared to controls. This finding may indicate less storage capacity for GABA and, therefore, possibly less GABA release and GABAergic inhibition in the midbrain of patients. After exocytosis, GABA can bind to the ionotropic receptor ($GABA_A$) and metabotropic receptor ($GABA_B$). One post-mortem study found significantly decreased mRNA levels of $GABA_A$ receptor alpha subunits 1-3 (GABRA1-3) and 5 (GABRA5) in the SN of patients with schizophrenia compared to controls.[25] In contrast, no significant group differences were observed with regard to GABRA3 protein levels.

Taken together, these findings suggest reduced GABAergic neurotransmission in the SN in schizophrenia, with lower density of GABAergic synapses and lower VGAT mRNA levels. Also mRNA levels for $GABA_A$ receptor subunits are lower although it is not clear yet whether this also leads to reduced receptor expression. Antipsychotic treatment may partly revert the reduced GABAergic neurotransmission by increasing GAD levels, although this may not be the case for all antipsychotics. All these data are based on post-mortem findings and *in vivo* research of the GABAergic system with PET imaging and MRS may further support these findings and might give more insight in the effect of antipsychotics and illness duration. Reduced cortical

GABAergic neurotransmission in schizophrenia patients is indeed found in a study using [^{11}C]flumazenil PET combined with blocking the GABA membrane transporter GAT1, although there was no data reported on the SN.[87] This study is in line with the suggestion of reduced GABAergic transmission in the SN, where due to lower density of GABAergic synapses, less storage and subsequent release of GABA, there might be less inhibition of the nigrostriatal dopaminergic pathway contributing to hyperdopaminergia.

5. Neuroinflammatory processes contribute to abnormalities in nigral functioning

Previous research has reported elevated expression of cytokines and other mediators of inflammation in the periphery and brains of patients with schizophrenia.[88,89,90] These findings suggest a role of inflammation in schizophrenia (as reviewed by Khandaker et al. (2015)[91]). As inflammatory mediators, such as chemokines and cytokines, influence the maintenance, development, and functional properties of dopaminergic neurons in the midbrain,[92,93] inflammatory processes in the SN might affect the nigral dopaminergic and other signalling pathways. One post-mortem study found increased mRNA levels of pro-inflammatory cytokines (i.e., IL1β, IL6, TNF-α, IL6ST, and IL17RA) and an acute-phase protein (i.e., serpin family A member 3 [SERPINA3]) in the SN of patients with schizophrenia.[94] In a subsequent analysis, mRNA levels of the pro-inflammatory cytokines (expect IL1A) and SERPINA3 were found to be elevated in patients with a high inflammatory biotype (i.e., high expression of inflammatory transcripts in the midbrain) compared to patients with a low inflammatory biotype (i.e., low expression of inflammatory transcripts in the midbrain) and controls.

Multiple cells within the brain can produce cytokines, such as microglia, astrocytes, and other glial cells. The glial hypothesis of schizophrenia has become a prominent theory of cognitive impairment and proposes that initial perturbations in glial cells (particularly astrocytes) can result in anomalies in neurotransmitters and neurons, which are involved in the pathogenesis of schizophrenia.[95] Purves-Tyson et al. (2021)[94] reported no alterations in mRNA levels of multiple microglial markers in the SN (i.e., allograft inflammatory factor 1 [AIF1], cluster of differentiation 68 [CD68], human leukocyte antigen [HLA], and translocator protein [TSPO]). However, CD68 and TSPO mRNA were elevated in patients with a high compared to a low inflammatory biotype and compared to controls. Another post- mortem study found that the density of astrocytes was significantly decreased in the SN of antipsychotic-treated patients with schizophrenia compared to both patients with major depressive disorder and controls.[70] Since astrocytes eliminate excessive extracellular glutamate from the synaptic cleft, decreased astrocyte density is suggested to result in relatively higher synaptic levels of glutamate, which may contribute to the hyperexcitability of

dopaminergic synapses. In contrast, Purves-Tyson et al. (2021)[94] found higher glial fibrillary acidic protein (GFAP) mRNA levels, which is used to index astrocyte activity, in the SN of patients with schizophrenia compared to controls. Patients with a high inflammatory biotype had significantly higher GFAP mRNA levels compared to patients with a low inflammatory biotype and controls. These results may indicate a compensatory mechanism to counteract the decreased astrocyte density in a subgroup of patients.

During neuroinflammatory conditions, CD163+ macrophages enter the brain tissue. Multiple lines of research suggest that macrophages also infiltrate the brain in schizophrenia.[96,97] Specifically, Purves-Tyson et al. (2020)[98] found increased macrophage density (i.e., CD163+ cell density), as well as, higher levels of macrophage markers (i.e., intracellular adhesion molecule 1 [ICAM1] mRNA, CD163 mRNA and protein expression, and fibronectin 1 mRNA) in the SN of patients with schizophrenia and a high inflammatory biotype compared to controls. This increase in macrophage markers appears to be related to an increase in complement synthesis, as elevated nigral C1qA, C3, and C4 complement mRNA levels were also found in patients with a high inflammatory status compared to controls.[98] This is of relevance since microglia regulate synaptic pruning via the complement pathway and as previously described in the section on GABAergic alterations, there is reduced GABAergic synaptic density which may be a result of aberrant synaptic pruning.[82] However, no corresponding increases in C3 and C4 complement protein levels were found.[98] In conclusion, as schizophrenia is a heterogeneous disease, alterations in nigral immune-related transcripts, complement synthesis, and markers of microglia and macrophages might be present in subgroups of patients and tend to be elevated particularly in patients with a high inflammatory biotype. Future research is needed to unravel the link between these alterations and the well-known dysregulation of dopaminergic neurotransmission in schizophrenia. The finding that astrocyte density is decreased in the SN in schizophrenia may result in higher synaptic levels of glutamate, and might thereby directly affect the nigrostriatal pathway through increased excitation.

Table 1. Studies examing different molecular aspects of nigral functioning in patients with schizophrenia.

Source	Method(s): constituent(s)	ROI	Sample size, No. (F:M)	Age, mean (SD), y	Antipsychotic mediation status, No.	Illness duration, mean (SD), y	Main result(s)
Allen et al. (2012)[33]	[18F]F-DOPA PET: dopamine synthesis	SN/ VTA	5 (2:3) UHR-t 16 (7:9) UHR-nt 14 (4:10) HC	UHR-t: 26.32 (4.22) UHR-nt: 25.51 (5.81) HC: 25.40 (3.60)	AP naïve (5 UHR-t, 14 UHR-nt) AP free (2 UHR-nt)	NA	↑↑ Dopamine synthesis in UHR-t vs UHR-nt = Dopamine synthesis in UHR-t vs HC
Arakawa et al. (2009)[50]	[11C]-PE2I PET: DAT binding	SN	8 (2:6) SCZ 12 (2:10) HC	SCZ: 36.50 (9.50) HC: 33.20 (12.00)	AP naïve (2 SCZ) AP free[a] (6 SCZ)	SCZ: 32.10 (42.80)[b]	= DAT binding in SCZ vs HC
Artiges et al. (2017)[49]	[11C]-PE2I PET: DAT binding	Left and right SN/ VTA	21 (0:21) SCZ 30 (0:30) HC	SCZ: 34.19 (10.23) HC: 30.17 (9.65)	AP naïve (1 SCZ) On AP (20 SCZ)	SCZ: 13.57 (9.25)	↑↑ Left and right DAT binding in SCZ vs HC
Cassidy et al. (2019)[39]	NM-MRI: NM	SN	33 (10:23) SCZ 30 (12:18) HC	SCZ: 33.90 (2.20)[e] HC: 34.00 (2.20)[e]	AP naïve[f] (17 SCZ) AP free[g] (16 SCZ)	NR	= NM in SCZ vs HC
Elkashef et al. (2000)[28]	[18F]F-DOPA PET: dopamine synthesis	SN/ VTA	10 (2:8) SCZ on medication 9 (2:7) SCZ off medication 13 (5:8) HC	SCZ on medication: 39.30 (8.70) SCZ off medication: 33.30 (7.90) HC: 34.70 (10.75)	AP free[h] (9 SCZ) On AP (10 SCZ)	SCZ on medication: 19.60 (7.80) SCZ off medication: 15.00 (8.40)	= Dopamine synthesis in SCZ on medication vs SCZ off medication vs HC
Graff-Guerrero et al. (2009)[64]	[11C]-(+)-PHNO PET: $D_{2/3}$ receptor availability	SN	13 (4:9) SCZ 13 (4:9) HC	SCZ: 25.85 (5.90) HC: 26.85 (6.40)	AP naïve (10 SCZ) AP free[p] (3 SCZ)	NR	= $D_{2/3}$ receptor availability in SCZ vs HC

Table 1. (Continued).

Source	Method(s): constituent(s)	ROI	Sample size, No. (F:M)	Age, mean (SD), y	Antipsychotic mediation status, No.	Illness duration, mean (SD), y	Main result(s)
Howes et al. (2013)[18]	Immunohisto-chemistry: TH; [18F]F-DOPA PET: AADC activity	SN	Post-mortem cohort 12 (5:7) SCZ 13 (4:9) HC *In vivo* cohort 29 (3:26) SCZ 29 (7:22) HC	Post-mortem cohort SCZ: 60.10 (2.30)[e] HC: 51.90 (2.80)[e] *In vivo* cohort SCZ: 33.70 (10.60)[e] HC: 29.30 (7.50)[e]	Post-mortem cohort On AP (12 SCZ) *In vivo* cohort AP naïve (5 SCZ) AP free[i] (8 SCZ) On AP (16 SCZ)	NR	↑↑ TH staining in SCZ vs HC ↑↑ AADC activity in SCZ vs HC
Ichinose et al. (1994)[23]	RT-PCR: TH mRNA, AADC activity	SN	8 (2:6) SCZ 12 (6:6) HC	SCZ: 67.00 (6.00)[e] HC: 71.00 (3.00)[e]	NR	NR	= TH mRNA in SCZ vs HC = AADC activity in SCZ vs HC
Jauhar et al. (2017)[27]	[18F]F-DOPA PET: AADC activity	SN	16 (2:14) SCZ 22 (8:14) HC	SCZ: 26.31 (4.40) HC: 24.45 (4.54)	AP naïve (11 SCZ) AP free[i] (3 SCZ) On AP (2 SCZ)	SCZ: 24[b,c,d]	= AADC activity in SCZ vs HC
Joo et al. (2018)[62]	[18F]fallypride PET: $D_{2/3}$ receptor availability	Left and right SN	16 (10:6) SCZ 17 (9:8) HC	SCZ: 36.90 (11.40) HC: 32.30 (9.50)	On AP (16 SCZ)	SCZ: 6.50 (3.70)	↓↓ Left and right $D_{2/3}$ receptor availability in SCZ vs HC
Kegeles et al. (2010)[65]	[18F]fallypride PET: $D_{2/3}$ receptor availability	SN	21 (7:14) SCZ 22 (5:17) HC	SCZ: 31.00 (12.00) HC: 26.00 (6.00)	AP naïve (5 SCZ) AP free[f] (16 SCZ)	NR	= $D_{2/3}$ receptor availability in SCZ vs HC
Kumakura et al. (2007)[29]	[18F]F-DOPA PET: AADC activity	Mid-brain	8 (0:8) SCZ 15 (0:15) HC	SCZ: 37.30 (6.30) HC: 37.30 (6.40)	AP naïve (3 SCZ) AP free[a] (5 SCZ)	NR	↑↑ AADC activity in SCZ vs HC

Table 1. (Continued).

Source	Method(s): constituent(s)	ROI	Sample size, No. (F:M)	Age, mean (SD), y	Antipsychotic mediation status, No.	Illness duration, mean (SD), y	Main result(s)
Kessler et al. (2009)[61]	[^{18}F]fallypride PET: $D_{2/3}$ receptor availability	Left and right SN/ VTA	11 (5:6) SCZ 11 (6:5) HC	SCZ: 30.50 (8.00) HC: 31.60 (9.20)	AP naïve (4 SCZ) AP free[g] (7 SCZ)	NR	↑↑ Left and right $D_{2/3}$ receptor availability in SCZ vs HC
Mabry et al. (2019)[74]	Immunohisto-chemistry: VGLUT1, VGLUT2, and GAD67; Electron microscopy: density of glutamatergic and GABAergic synapses	SN	Immunohistoche-mistry cohort 6 (2:4) SCZ 5 (2:3) HC Electron microscopy cohort 11 (3:8) SCZ 8 (2:6) HC	Immunohistoche-mistry cohort SCZ: 53.80 (13.70) HC: 51.20 (11.40) Electron microscopy cohort SCZ: 50.60 (10.60) HC: 43.40 (15.30)	Immunohistoche-mistry cohort AP free[a] (3 SCZ) On AP (2 SCZ) NR (1 SCZ) Electron miscroscopy cohort On AP (11 SCZ)	NR	= VGLUT1 and VGLUT2 levels in SCZ vs HC = GAD67 in SCZ vs HC = Density of glutamatergic synapses in SCZ vs HC ↓ Density of GABAergic synapses in SCZ vs HC
Mueller et al. (2004)[21]	*In situ* hybridization: TH, NR1, NR2A-D, NR3A, GluR1-7, and KA1-2 mRNA, and PSD-93, PSD-95, NF-L, SAP102, and Yotiao protein expression	SNc	17 (9:8) SCZ 7 (5:2) HC	SCZ: 73.88 (NR) HC: 76.00 (NR)	AP free[k] (5 SCZ) On AP (12 SCZ)	NR	↑↑ TH and GluR5 mRNA in SCZ vs HC ↑ NR1 mRNA in SCZ vs HC = NR2A-D, NR3A, GluR1-4, GluR6-7, and KA1-2 mRNA in SCZ vs HC = PSD-93, PSD-95, NF-L, SAP102, and Yotiao protein expression in SCZ vs HC

Table 1. (Continued).

Source	Method(s): constituent(s)	ROI	Sample size, No. (F:M)	Age, mean (SD), y	Antipsychotic mediation status, No.	Illness duration, mean (SD), y	Main result(s)
Owen et al. (1984)[60]	[^{3}H]-spiperone PET: $D_{2/3}$ receptor availability	SN	10 (5:5) SCZ AP free 9 (5:4) SCZ On AP 9 (4:5) HC	SCZ AP free: 71.20 (11.90) SCZ On AP: 63.70 (16.80) HC: 73.40 (12.70)	AP free[l] (10 SCZ) On AP (9 SCZ)	NR	↑ $D_{2/3}$ receptor availability in SCZ AP free vs HC ↑ $D_{2/3}$ receptor availability in SCZ On AP vs HC
Perez-Costas et al. (2012)[24]	*In situ* hybridization: TH mRNA; Western blot: TH protein expression	SN/ VTA	6 (2:4) SCZ 5 (2:3) HC	SCZ: 54.33 (14.73) HC: 54.80 (9.28)	On AP (6 SCZ)	NR	= TH mRNA in SCZ vs HC ↓ TH protein levels in SCZ vs HC
Purves-Tyson et al. (2021)[25]	Fluidigm qPCR: TH, GAD1, VGAT, GABRA1-3, GABRA-5, and DAT mRNA; Western blotting: GAD65/67 and GABRA3 protein levels	SN	mRNA cohort 28 (9:19) SCZ 28 (8:20) SCZ Protein cohort 26 (10:16) SCZ 28 (9:19) HC	mRNA cohort SCZ: 51.36 (26-67)[m] HC: 50.54 (22-67)[m] Protein cohort SCZ: 52.29 (26-67)[m] HC: 52.21 (22-69)[m]	mRNA cohort On AP (28 SCZ) Protein cohort On AP (26 SCZ)	mRNA cohort SCZ: 28.31 (12.72) Protein cohort SCZ: 29.12 (13.02)	↓ TH mRNA in SCZ vs HC ↓↓ GAD1 and VGAT mRNA in SCZ vs HC ↓↓ DAT mRNA in SCZ vs HC ↓↓ GABRA1-3 mRNA in SCZ vs HC ↓ GABRA5 mRNA in SCZ vs HC ↓ GAD65/67 protein levels in SCZ vs HC = GABRA3 protein levels in SCZ vs HC
Reid et al. (2013)[80]	^{1}H-MRS: Glx/Cr, Glx/NAA	SN	35 (9:26) SCZ[n] 22 (9:13) HC	SCZ: 37.90 (12.00) HC: 37.90 (12.40)	On AP (35 SCZ)	SCZ: 17.20 (11.20)	= Glx/Cr in SCZ vs HC ↑ Glx/NAA in SCZ vs HC

Table 1. (Continued).

Source	Method(s): constituent(s)	ROI	Sample size, No. (F:M)	Age, mean (SD), y	Antipsychotic mediation status, No.	Illness duration, mean (SD), y	Main result(s)
Purves-Tyson et al. (2017)[22]	Western blot: TH and DAT protein levels; RT-PCR: TH, DAT, AADC, VMAT-2, and D_2/D_3 receptor[q] mRNA	SN	mRNA cohort 28 (9:19) SCZ 29 (9:20) HC Protein cohort 26 (10:16) SCZ 27 (8:19) HC	mRNA cohort SCZ: 51.40 (26-67)[m] HC: 51.20 (22-69)[m] Protein cohort SCZ: 52.29 (26-67)[m] HC: 52.21 (22-69)[m]	mRNA cohort On AP (28 SCZ) Protein cohort On AP (26 SCZ)	mRNA cohort SCZ: 28.31 (12.72) Protein cohort SCZ: 29.12 (13.02)	= TH and DAT protein levels in SCZ vs HC ↓↓ DAT mRNA in SCZ vs HC ↓ VMAT-2 and DRD2S mRNA in SCZ vs HC = TH, AADC, DRD2L, DRD2Longer, DRD3 full-length, and DRD3 non-functional mRNA in SCZ vs HC
Purves-Tyson et al. (2021)[94]	qPCR: IL1β, IL6, IL8, IL18, TNF-α, IL6ST, IL1A, IL17RA, and SERPINA3 mRNA; Immunohisto-chemistry: AIF1, CD68, GFAP, HLA, and TPSO	SN	28 (9:19) SCZ 29 (9:20) HC	SCZ: 51.40 (26-67)[m] HC: 51.20 (22-69)[m]	On AP (28 SCZ)	SCZ: 28.31 (12.72)	↑↑ IL1β, IL6, IL17RA, and SERPINA3 mRNA in SCZ vs HC ↑ TNF-α and IL6ST mRNA in SCZ vs HC = IL8, IL18, and IL1A mRNA in SCZ vs HC = AIF1, CD68, HLA, TSPO mRNA in SCZ vs HC ↑↑ GFAP mRNA in SCZ vs HC
Rice et al. (2016)[71]	Immunohistoch-emistry: number of dopaminergic neurons, total neurons, and their ratio	SN/ VTA	6 (2:4) SCZ 7 (1:6) HC	SCZ: 53.80 (15.04) HC: 53.43 (10.92)	On AP (6 SCZ)	NR	= Number of dopaminergic neurons, total neurons, and their ratio in SCZ vs HC

Table 1. (Continued).

Source	Method(s): constituent(s)	ROI	Sample size, No. (F:M)	Age, mean (SD), y	Antipsychotic mediation status, No.	Illness duration, mean (SD), y	Main result(s)
Purves-Tyson et al. (2020)[98]	RT-PCR: ICAM1, CD163, FN1, C1qA, C3, and C4 mRNA; Western blotting: CD163, C3, and C4 protein expression; DAB immunohisto-chemistry: CD163+ denisty	SN	mRNA cohort 15 (4:11) SCZ LIS 13 (5:8) SCZ HIS 28 (8:20) HC Protein cohort 13 (4:9) SCZ LIS 12 (5:7) SCZ HIS 26 (7:19) HC	mRNA cohort SCZ LIS: 48.27 (30-64)[m] SCZ HIS: 54.92 (26-67)[m] HC: 50.54 (22-67)[m] Protein cohort SCZ LIS: 49.31 (30-64)[m] SCZ HIS: 56.17 (26-67)[m] HC: 51.19 (22-67)[m]	mRNA cohort On AP (15 SCZ LIS and 13 SCZ HIS) Protein cohort On AP (13 SCZ LIS and 12 SCZ HIS)	mRNA cohort SCZ LIS: 26.43 (11.65) SCZ HIS: 31.62 (13.93) Protein cohort SCZ LIS: 25.92 (11.30) SCZ HIS: 32.33 (14.29)	↑↑ ICAM1, CD163, C1qA, C3, and C4 mRNA in SCZ HIS vs SCZ LIS/HC ↑↑ CD163 protein expression in SCZ HIS vs HC ↑ CD163 protein expression in SCZ HIS vs SCZ LIS ↑↑ FN1 mRNA in SCZ HIS vs HC ↑ CD163+ denisty in SCZ HIS vs HC = C3 protein expression in SCZ HIS vs SCZ LIS/HC ↑↑ C4 protein expression in SCZ LIS vs SCZ HIS ↑ C4 protein expression in SCZ LIS vs HC
Sasaki et al. (2010)[36]	NM-MRI: NM	SNc	23 (8:15) SCZ 23 (13:10) HC	SCZ: 44.90 (13.60) HC: 47.00 (16.90)	On AP (23 SCZ)	SCZ: 19.40 (10.90)	= NM in SCZ vs HC
Schoon-over et al. (2017)[19]	Western blotting: TH, VGLUT1, VGLUT2, and GAD67 protein levels	Caudal SN	13 (3:10) SCZ 12 (3:9) HC	SCZ: 42.20 (13.10) HC: 50.40 (15.70)	AP free[a] (4 SCZ) On AP (9 SCZ)	SCZ: 18.60 (9.80)	↑↑ TH and GAD67 protein levels in SCZ vs HC = VGLUT1 and VGLUT2 levels in SCZ vs HC

Table 1. (Continued).

Source	Method(s): constituent(s)	ROI	Sample size, No. (F:M)	Age, mean (SD), y	Antipsychotic mediation status, No.	Illness duration, mean (SD), y	Main result(s)
Schoon-over et al. (2020)[101]	Western blotting: ATP7A N and C terminus, ATP7B, extracellular and transmembrane CTR1, and dysbindin 1A and 1B/C protein levels; ICP-MS: copper content	SN	Western blot cohort 15 (4:11) SCZ 11 (2:9) HC Tissue copper content cohort 14 (3:11) SCZ 14 (3:10) HC	Western blot cohort SCZ: 42.10 (12.20) HC: 50.90 (16.30) Tissue copper content cohort SCZ: 41.20 (13.00) HC: 39.40 (10.80)	Western blot cohort AP free[a] (4 SCZ) On AP (11 SCZ) Tissue copper content cohort AP free (4 SCZ) On AP (10 SCZ)	Western blot cohort SCZ: 18.60 (9.30) Tissue copper content cohort SCZ: 19.10 (9.30)	↑ ATP7A C terminus protein levels in SCZ vs HC ↓↓ Transmembrane CTR1 protein levels in SCZ vs HC ↓ Dysbindin 1B/C protein levels in SCZ vs HC = ATP7A N terminus, ATP7B, extracellular CTR1, and dysbindin 1A protein levels in SCZ vs HC ↓↓ Copper content in SCZ vs HC
Shibata et al. (2008)[37]	NM-MRI: NM	SNc	20 (7:13) SCZ 34 (17:17) HC	SCZ: 44.60 (12.40) HC: 43.80 (11.20)	On AP (20 SCZ)	SCZ: 18.30 (11.70)	↑ NM in SCZ vs HC
Slifstein et al. (2015)[44]	[^{11}C]-FLB457 PET + amphetamine challenge: dopamine release	SN/ VTA	20 (10:10) SCZ[n] 21 (11:10) HC	SCZ: 33.10 (10.20) HC: 32.60 (8.10)	AP naïve (6 SCZ) AP free[g] (14 SCZ)	SCZ: 13.20 (11.30)	= Dopamine release in SCZ vs HC
Spokes et al. (1980)[84]	Radiochemical method: GAD activity	SN	42 (20:22) SCZ 52 (22:30) HC	SCZ: 59.80 (18.10) HC: 64.40 (21.60)	On AP (42 SCZ)	NR	= GAD activity in SCZ vs HC

Table 1. (Continued).

Source	Method(s): constituent(s)	ROI	Sample size, No. (F:M)	Age, mean (SD), y	Antipsychotic mediation status, No.	Illness duration, mean (SD), y	Main result(s)
Suridjan et al. (2013)[66]	[^{11}C]-(+)-PHNO PET: $D_{2/3}$ receptor availability	SN	13 (3:10) SCZ 12 (5:7) HC	SCZ: 23.38 (4.60) HC: 26.10 (3.80)	AP naïve (13 SCZ)	NR	= $D_{2/3}$ receptor availability in SCZ vs HC
Toru et al. (1988)[20]	$^{14}CO_2$-trapping method: TH protein levels; Spectrofluorometry: glutamate and GABA protein levels	SN	14 (5:9) SCZ 10 (3:7) HC	SCZ: 57.90 (3.50)[e] HC: 66.70 (2.70)	AP naïve (1 SCZ) AP free (6 SCZ) On AP (7 SCZ)	NR	↑↑ TH protein levels in SCZ vs HC = Glutamate and GABA protein levels in SCZ vs HC
Tuppurainen et al. (2006)[63]	[^{123}I]-epidepride SPECT	Midbrain	6 (4:2) SCZ 7 (3:4) HC	SCZ: 33.00 (14.00) HC: 31.00 (9.00)	AP naïve (6 SCZ)	SCZ: 11.00[b] (1.00-36.00)[m]	↓ $D_{2/3}$ receptor availability in SCZ vs HC
Tseng et al. (2018)[43]	[^{11}C]-(+)-PHNO PET + psychosocial stress challenge: dopamine release	SN	9 (3:6) SCZ[o] 25 (12:13) HC	SCZ: 24.11 (5.33) HC: 25.12 (4.45)	AP naïve (9 SCZ)	NR	↑↑ Dopamine release in SCZ vs HC
Watanabe et al. (2014)[38]	NM-MRI: NM	SNc	52 (25:27) SCZ 52 (25:27) HC	SCZ: 35.10 (13.30) HC: 34.60 (13.70)	NR	SCZ: 10.40 (10.90)	↑↑ NM in SCZ vs HC
Zubieta et al. (2001)[34]	(+)-α-[^{11}C]DTBZ PET: VMAT-2 binding	Ventral brainstem	12 (4:8) SCZ 15 (6:9) HC	SCZ: 36.00 (11.00) HC: 38.00 (11.00)	On AP (12 SCZ)	NR	↑ VMAT-2 binding in SCZ vs HC
Yamashita et al. (2016)[40]	NM-MRI: NM	SNc	14 (3:11) SCZ 22 (8:14) HC	SCZ: 37.00 (27-63)[m] HC: 40.00 (25-59)[m]	On AP (14 SCZ)	SCZ: 9.50 (3.00-32.00)[m]	= NM in SCZ vs HC

Table 1. (Continued).

Source	Method(s): constituent(s)	ROI	Sample size, No. (F:M)	Age, mean (SD), y	Antipsychotic mediation status, No.	Illness duration, mean (SD), y	Main result(s)
Williams et al. (2014)[70]	Histochemistry: nuclear length, nucleolar volume, nuclear area, and somal cross-sectional area of dopaminergic neurons and oligodendrocyte density; Immunohistochemistry: astrocyte denisty	SN	12 (5:7) SCZ 13 (4:9) HC	SCZ: 60.10 (2.30) HC: 51.90 (2.80)	NR	NR	↑ Nuclear length, nucleolar volume, and nuclear area of dopaminergic neurons in SCZ vs HC = Somal cross-sectional area of dopaminergic neurons in SCZ vs HC ↓ Astrocyte denisty in SCZ vs HC

Abbreviations: AADC, Aromatic L-amino acid decarboxylase; AIF1, allograft inflammatory factor 1; AP, antipsychotic medication; CD68, cluster of differentiation 68; Cr, creatine; CT1, copper transporter-1; DAB, 3,3 diaminobenzidine; DAT, dopamine transporter; DRD2, dopamine receptor D_2; DRD3, dopamine receptor D_3; FN1, fibronectin 1; GABA, γ-aminobutyric acid; GAD, glutamate decarboylase; GABRA, $GABA_A$ receptor alpha subunit; GFAP, glial fibrillary acidic protein; Glx, glutamate and glutamine; HC, (healthy) controls; HIS, high inflammatory status; HLA, human leukocyte antigen; ICAM1, intracellular adhesion molecule 1; ICP-MS, inductively-coupled plasma mass spectrometry; IL6ST, IL6 signal transducer; LIS, low inflammatory status; mRNA, messenger ribonucleic acid; NA, not applicable; NAA, N-acetyl-aspartate; NM, neuromelanin; NM-MRI, neuromelanin-sensitive magnetic resonance imaging; NR, not reported; PET, positron emission tomography; qPCR, quantitative polymerase chain reaction; ROI, region of interest; RT-PCR, reverse transcription polymerase chain reaction; SCZ, patient with schizophrenia; SERPINA3, serpin family A member 3; SN, substantia nigra; SNc, substantia nigra pars compacta; SN/VTA, a midbrain region including the SN and ventral tegmental area; SPECT, single-photon emission computerized tomography; TH, tyrosine hydroxylase; TNF-α, tumor necrosis factor α; TSPO, translocator protein; UHR-nt, ultra-high-risk individuals who did not develop a psychotic disorder; UHR-t, ultra-high-risk individuals who developed a psychotic disorder; VGAT, vesicular GABA transporter; VGLUT, vesicular glutamate transporter; VMAT-2, vesicular monoamine transporter 2; ^{1}H-MRS, proton magnetic resonance spectroscopy; ↑↑, significantly increased with $p < 0.01$; ↑, significantly increased with $p \leq 0.05$; ↓↓, significantly decreased with $p < 0.01$; ↓, significantly decreased with $p \leq 0.05$; =, no significant difference.

Table 1. (Pages 132-140).

[a]For at least six months. [b]Illness duration is expressed in months instead of years. [c]The median is reported instead of the mean. [d]Interquartile range is reported instead of the standard deviation. [e]Standard error is reported instead of the standard deviation. [f]Lifetime exposure was less than six weeks and patients did not use antipsychotic medication in the past three weeks. [g]For at least three weeks. [h]For at least thirty-three days. [i]For at least three months. [j]For at least six weeks in case of oral medication and for at least six months in case of depot medication. [k]For at least six weeks. [l]For at least one year. [m]The range is reported instead of the standard deviation. [n]The patient sample also consisted of patients diagnosed with schizoaffective disorder. [o]The patient sample also consisted of patients diagnosed with schizophreniform disorder. [p]For at least two weeks. [q]DRD2S, DRD2L, DRD2Longer, DRD3 full-length, and DRD3 non-functional.

6. Disrupted copper homeostasis in the substantia nigra

The copper hypothesis of schizophrenia is a relatively old theory that proposes that excess tissue copper can cause schizophrenia. Although elevated copper in the blood of patients has been reported in many studies (as reviewed by Bowman & Lewis (1982)[99]), the hypothesis has never been convincingly refuted nor demonstrated. As copper can affect the production, as well as, the breakdown of dopamine, copper might also be relevant for the signalling pathways within the SN. Endothelial cells at the blood-brain barrier take up copper from the bloodstream via the copper transporter-1 (CTR1).[100] Subsequently, copper is released into the brain parenchyma via the copper transporter ATP7A.[100] One post-mortem study compared dysbindin (i.e., dysbindin isoforms 1A and 1B/C), a protein which controls copper transport, and copper transport protein (i.e., ATP7A, ATP7B, and CTR1) expression and copper content in the copper-rich SN between patients with schizophrenia and controls.[101] ATP7A C terminus protein levels were increased, transmembrane CTR1 and dysbindin 1B/C protein levels were decreased, and ATP7A N terminus and extracellular CTR1, dysbindin 1A and ATP7B protein levels were unaltered in the SN of patients with schizophrenia compared to controls. Additional post-hoc analyses revealed significantly lower N terminus ATP7A protein levels in unmedicated patients compared to controls and medicated patients, whereas C terminus ATP7A protein levels were increased in medicated patients compared to controls. Finally, a reduced amount of nigral copper was observed in patients with schizophrenia compared to controls. Medicated patients demonstrated significantly lower levels of copper than controls. This was not the case for unmedicated patients. Although we cannot rule out the possibility that antipsychotic medication modulates copper homeostasis, there may be a copper-deficient state within the SN of patients. So far, it remains poorly understood how disrupted copper homeostasis might be related to the pathology of schizophrenia. Some researchers have hypothesized that the blood-brain barrier is leaky in schizophrenia.[102] This potentially results in the uncontrolled leaking of copper into the brain, which incorrectly triggers a signal of excess copper. Subsequently, CTR1 prevents additional copper transport.[101] Copper-

decreasing experimental manipulation, through for example administering the copper chelator cuprizone, has been shown to result in increased dopamine levels (via inhibition of dopamine-β-hydroxylase [DBH]), decreased oligodendrocytic protein expression, and demyelination in animals.[103,104] Further research is needed to validate these findings in humans and to clarify the association between a copper-deficient state and schizophrenia.

7. Summary of molecular alterations in the substantia nigra

The available literature suggests that molecular alterations occur in the SN of patients with schizophrenia. These changes entail alterations in dopaminergic, glutamatergic, and GABAergic functioning, as well as, the functioning of the immune system and copper homeostasis (Figure 2). Overall, there is some evidence for hyperdopaminergia in the SN of patients (i.e., increased AADC activity in antipsychotic-free/-naïve patients and elevated neuromelanin accumulation). These findings are in line with the well-established finding of striatal hyperdopaminergia in schizophrenia (i.e., increased dopamine synthesis capacity and dopamine release in the striatum) and show that the hyperdopaminergia is not only present in the striatum. Within the SN, the hyperdopaminergia might be compensated by reduced functioning of VMAT-2, which subsequently could limit the release of dopamine. In addition, the hyperdopaminergia could theoretically be compensated by increased functioning of DAT. However, the current literature does not support such a compensatory mechanism. Hyperdopaminergia in the SN of patients with schizophrenia might be secondary to alterations in other molecular aspects of the SN. Reduced GABAergic function in the SN (i.e., lower density of GABAergic synapses, lower VGAT mRNA levels, and lower mRNA levels for $GABA_A$ receptor subunits) may contribute to the nigrostriatal hyperdopaminergia by providing reduced inhibition. Due to antipsychotic use, nigral GAD levels, and potentially other aspects of GABAergic functioning in the SN, might change to increase the inhibition of dopaminergic neurons and thereby compensate for the hyperdopaminergic state. Accordingly, evidence has emerged that the tail of the VTA may act as a GABAergic brake to inhibit dopaminergic neurons of the SNc.[105] Therefore, GABAergic dysregulation in the VTA might also contribute to the presynaptic hyperdopaminergia in the SN. Increased glutamatergic excitation of the SN may also contribute to nigrostriatal hyperdopaminergia. The increased glutamatergic neurotransmission may be due to increased NR1 and Glur5 mRNA levels, increased VGLUT2 levels in antipsychotic-free patients (which might be indicative of elevated glutamate release), and reduced density of astrocytes (since astrocytes remove extracellular glutamate this might result in prolonged neurotransmission). As increased GFAP mRNA levels have also been reported in schizophrenia, this might be a compensatory mechanism to counteract the decreased astrocyte density. Finally,

nigrostriatal hyperdopaminergia could also be related to alterations in the immune system and copper functioning, through the influence of inflammatory mediators on the functioning of dopaminergic neurons in the midbrain[92,93] or due to a possible inability to break down monoamines in a copper-dependent way, respectively.

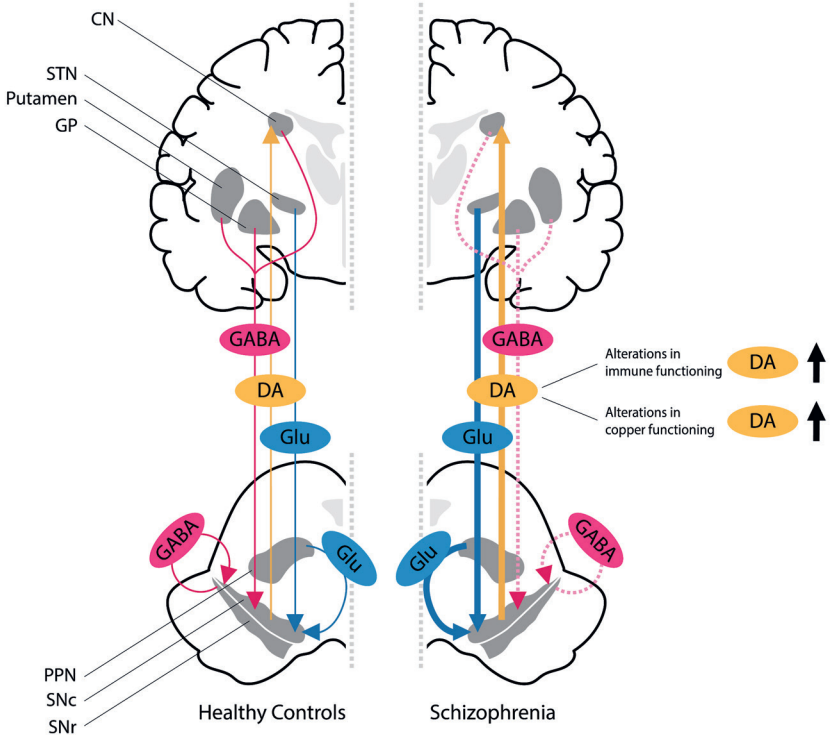

Figure 2. Substantia nigra pathology in schizophrenia. Hyperdopaminergia in the substantia nigra of patients with schizophrenia is most likely a manifestation of a hyperactive dopaminergic nigrostriatal pathway compared to HC. The nigrostriatal pathway projects from the SNc to the associative striatum. GABAergic projections from the neostriatum, GP, and SNr to the SNc might provide reduced inhibition in patients with schizophrenia compared to HC and thereby contribute to the nigrostriatal hyperdopaminergia. In addition, the nigrostriatal hyperdopaminergia could also be a result of increased glutamatergic excitation of the SNc compared to HC via glutamatergic projections that originate in the PPN and STN. Finally, molecular changes with regard to immune and copper functioning within the substantia nigra could also contribute to the nigrostriatal hyperdopaminergia.

Abbreviations: DA, dopamine; CN, caudate nucleus; GABA, γ-minobutyric acid; Glu, glutamate; GP, globus pallidus; PPN, pedunculopontine nucleus; SNc, substantia nigra pars compacta; SNr, substantia nigra pars reticulata; STN, subthalamic nucleus.

8. Additional insights into the striatonigrostriatal circuit in schizophrenia

This review focused on human studies. Due to practical reasons, it is challenging and often impossible to study the interactions between the different regions of the striatonigrostriatal (SNS) circuit in the living brain of humans. Animal circuit studies,

however, can provide additional insight into the functions of the SNS circuit. Haber et al. (2000)[9] showed in primates that the SNS projection from the ventromedial, central, and dorsolateral striatal subregions contain three SN components: (1) a dorsal region of nigrostriatal projecting cells, (2) a central group of both nigrostriatal projecting cells and their reciprocal striatonigral terminal fields, and (3) a ventral region that contains a striatonigral projection but not its reciprocal nigrostriatal projection. Information can travel through the SNS circuit in multiple ways: (1) via direct/reciprocal connections and (2) via indirect/nonreciprocal connections. Midbrain projections from the shell of the striatum target both the ventromedial SNc and the VTA. Projections from the VTA back to the shell of the striatum form the reciprocal SNS loop. Midbrain projections from the medial SN feedforward to the core of the striatum and form the first part of the nonreciprocal connections (also referred to as spiral). Subsequently, the spiral continues through the SNS circuit with pathways originating in the core of the striatum and projecting more dorsally. In this way, ventral striatal regions affect dorsal striatal regions via these spiralling SNS projections.[9] In addition, Lerner et al. (2015)[106] implied that, besides a ventral-to-dorsal route, there might also be a lateral-to-medial information flow through the circuit. Haber et al. (2000)[9] proposed the following model of the synaptic interactions of the SNS projections in the reciprocal and non-reciprocal loops. The reciprocal component of the SNS circuit terminates directly on an SNc dopaminergic cell. Activation of this component results in inhibition. The nonreciprocal component, in contrast, terminates indirectly on an SNc dopaminergic cell, i.e., via a GABAergic interneuron, and activation of this component results in disinhibition and facilitation of the dopaminergic cell burst firing. Consequently, each part of the spiral sends an inhibitory feedback response, but also facilitates the transfer of information to the next step of the spiral via disinhibition. The model of Haber et al. (2000)[9] is supported by rodent studies which demonstrate that stimulation of the striatum can result in an elevation of dopamine firing via inhibition of GABAergic interneurons.[107-110] This is in line with the human data summarized in this review and stresses that an imbalance between inhibition and disinhibition of the SNS circuit might be important in the pathology of schizophrenia. Indeed, the GABAergic projections from the striatum to SNc as depicted in Figure 2 might reflect the reciprocal component of the SNS circuit,[9,106] which seems to be downregulated in patients with schizophrenia. The GABAergic interneuron of the nonreciprocal component might correspond to the GABAergic connection between the SNr and SNc as depicted in Figure 2. These interneurons are innervated by GABAergic projections from the striatum (perhaps as part of the nonreciprocal component) and the globus pallidus, as well as by glutamatergic projections from the subthalamic nucleus.[111] Possibly, the function of these non-reciprocal GABAergic interneurons is also reduced in schizophrenia, due to the reduced density of GABAergic synapses and consequently reduced storage and

exocytosis of GABA, as described in the human studies reviewed above. Reduction of the GABAergic inhibition of the dopaminergic neurons in schizophrenia likely results in the disinhibition of dopaminergic striatonigral projections to the striatum. Several animal models have been developed in an attempt to understand the neurobiological basis of schizophrenia, but to our knowledge, the SNS circuit has not yet been examined within this context. Future studies that address animal models for schizophrenia which focus on the SNS circuit would be necessary to translate the findings on reciprocal and nonreciprocal SNS connections and the role of glutamatergic and GABAergic inputs to the SN to patients with schizophrenia.

9. Limitations and future directions

Importantly, some limitations of the included studies and suggestions for future studies can be delineated. First of all, only a limited number of findings (primarily the dopaminergic findings) have been replicated, whereas the findings on GABAergic, glutamatergic, as well as, other molecular aspects of the SN, are based on a limited number of studies. In addition, some molecular aspects of the SN, such as nigral VGLUT3 levels, have never been investigated in patients with schizophrenia or only in underpowered cohorts. Additional research is therefore urgently needed to validate and expand the previous findings. Secondly, this review has discussed numerous post-mortem studies. The patient samples in these studies have often been treated with antipsychotic medication for extended periods. Therefore, the effect of antipsychotic medication on post-mortem findings can often not be excluded. Although post-mortem studies are essential to guide theories on schizophrenia pathology, support for these findings at an earlier disease stage and without chronic antipsychotic use *in vivo* is crucial. Furthermore, multimodal imaging techniques, such as combined PET and ^{1}H-MRS, or other techniques, such as fMRI and pharmacological MRI, could be used to study how alterations in multiple neurotransmitters systems relate to each other in the same individual, as well as, how changes in neurotransmitter systems are related to functional abnormalities in the prefrontal-striatal-nigro circuit.[112,113] Thirdly, although imaging techniques such as PET and SPECT have enabled researchers to study dopaminergic functioning in the SN *in vivo*, the spatial resolution of these techniques is limited, especially for small brain structures such as the SN. Fortunately, new developments in MR imaging, such as NM-MRI and pharmacological MRI, offer new opportunities to indirectly investigate the dopaminergic system, without radiation burden, with fewer costs, and within a shorter time frame. Additionally, since schizophrenia is a heterogeneous disease, it might be possible that the reported abnormalities in the SN are only present in a subgroup of patients. Previous research has suggested neurobiological differences between patients with schizophrenia that respond well (i.e., responders) compared to patients that do not respond adequately to antipsychotic

treatment (i.e., non-responders).[114] In line with this hypothesis, two subtypes of schizophrenia have been proposed: a hyperdopaminergic type A and a normodopaminergic type B, which are characterized by elevated striatal dopamine synthesis and normal presynaptic dopaminergic functioning in the striatum, respectively.[115] Conflicting findings might therefore be explained by different distributions of schizophrenia subtypes across studies. Neurobiological heterogeneity could also explain why some antipsychotic-naïve patients with schizophrenia have parkinsonism.[116,117] Those findings suggest the presence of a third subtype of schizophrenia with a hypoactive dopaminergic nigrostriatal pathway. In addition, as summarized in this review, there might also be schizophrenia subtypes with altered immune and/or copper functioning. Future research is needed to disentangle differences between multiple subgroups of patients. This might eventually contribute to the development of subgroup-specific treatment. Box 1 summarises limitations of current findings and future directions for research identified by this review.

10. Conclusions

This paper provides a comprehensive overview of molecular abnormalities in the SN of patients with schizophrenia, by addressing post-mortem and molecular imaging studies. Overall, there is some evidence for hyperdopaminergia in the SN of patients with schizophrenia. Reduced GABAergic inhibition, excessive glutamatergic excitation, as well as, alterations in other molecular aspects of the SN, such as immune functioning and copper homeostasis, could potentially underlie this nigrostriatal hyperdopaminergia. Importantly, these findings should be replicated and further investigated, as many studies consisted of small cohorts and may have been influenced by factors such as antipsychotic use and heterogeneity of patient cohorts. If replicated, some aspects of molecular functioning in the SN (e.g., neuromelanin concentrations) might provide important implications for future clinical practice as potential biomarkers or treatment targets.

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12. Author contributions

EvdG and MvdP conceived and designed the study. CvH, EvdG, IB, and MvdP designed the search strategy. CvH and IB did the literature search, selected the studies, and extracted the relevant information with support from EvdG and MvdP. CvH and IB synthesized the data and wrote the manuscript with support from EvdG, JB, JPS, LdH, MvdP, and TvA. All the authors critically reviewed the manuscript for intellectual

content. All authors approved the final version of the manuscript for publication. EvdG supervised the project.

13. Declarations of interest

All authors declare no conflict of interest.

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Box 1. Limitations and future directions.

Limitations	Future directions
Many molecular aspects of nigral functioning in patients with schizophrenia are investigated by few and often small cohort studies. In addition, patients were often chronically ill and exposed to antipsychotic medication for extended periods.	Multiple large well-powered studies in different cohorts (i.e., first episode psychosis patients and chronic patients) are necessary to replicate and extend previous findings. These studies should take the effect of factors such as age, antipsychotic medication, and illness duration into account. This will eventually also allow the use of objective quantitative methods, such as meta-analyses, to analyse and summarize the findings of the individual studies.
Imaging techniques may be limited in their abilities to examine specific aspects of molecular functioning within the SN (i.e., PET and SPECT cannot distinguish between D_2 and D_3 receptor binding and ^{1}H-MRS cannot distinguish between intra- and extracellular concentrations of glutamate). This hampers our understanding of the precise mechanisms that underlie the (nigral) pathology of schizophrenia.	Efforts to improve and develop imaging techniques would enable detailed localization of alterations in different aspects of (nigral) molecular functioning.
Schizophrenia is a heterogeneous disease. Therefore, it might be possible that the reported abnormalities in the SN are only present in a subgroup of patients. Study cohorts are often very heterogeneous, which limits the possibility to examine subgroup differences.	Data of homogenous subgroups should be combined to unravel subgroup-specific alterations. This might eventually lead to subgroup-specific interventions.
Due to practical reasons (e.g., small size of SN), it is challenging and often impossible to study the interactions within the striatonigrostriatal (SNS) circuit in the living brain of humans.	Animal models which focus on the SNS circuit would be necessary to translate the findings on reciprocal and nonreciprocal connections to patients with schizophrenia.

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Chapter

Striatal dopamine synthesis capacity and neuromelanin in the substantia nigra: a multimodal imaging study in schizophrenia and healthy controls

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Abstract

[18F]F-DOPA PET is an established *in-vivo* method for investigating striatal dopamine synthesis capacity (DSC) and has demonstrated abnormalities in striatal DSC in schizophrenia. Neuromelanin-sensitive MRI (NM-MRI) is a promising, more accessible, tool that indirectly assesses dopaminergic functioning in the substantia nigra (SN). However, how [18F]F-DOPA PET and NM-MRI, as measures of nigrostriatal dopaminergic functioning, interrelate is still unknown. We hypothesize that NM-MRI signal in the SN is positively correlated with striatal DSC in patients with a schizophrenia spectrum disorder (SSD) and healthy controls (HC). We acquired NM-MRI and dynamic [18F]F-DOPA PET scans in 12 patients with SSD and 16 HC. In both groups, we assessed the correlation between nigral NM-MRI signal and DSC in the whole, associative, limbic, and sensorimotor striatum using voxelwise analyses within the SN. In HC, we found subsets of voxels within the SN where NM-MRI signal correlated negatively with DSC in the whole and limbic striatum. There were no significant associations between NM-MRI and DSC in the associative or sensorimotor striatum in HC and no significant associations in patients. These results show that NM-MRI signal and striatal DSC are negatively related in HC, but not in patients. Our results indicate that [18F]F-DOPA PET and NM-MRI reflect different aspects of dopaminergic functioning. The negative correlation in HC might be explained by vesicular monoamine transporter-2 (VMAT-2) functioning. A lack of a correlation in patients might be due to the small sample size, effects of symptom severity or antipsychotic medication.

1. Introduction

[^{18}F]F-DOPA positron emission tomography (PET) is a well-established method for investigating striatal dopamine synthesis capacity (DSC). [^{18}F]F-DOPA PET studies have repeatedly demonstrated elevated striatal DSC (i.e., indicating striatal hyperdopaminergia), specifically in the associative striatum of patients with schizophrenia.[1,2] PET imaging leads to (limited) radiation exposure to the patient and can be time-consuming and expensive. Therefore, new imaging methods have been developed to assess the dopaminergic system.

One promising tool that indirectly assesses dopaminergic functioning in the substantia nigra (SN) is neuromelanin-sensitive MRI (NM-MRI).[3] Neuromelanin is a black, insoluble pigment, that primarily accumulates in the dopaminergic neurons of the SN pars compacta (SNc).[4] The deposition of neuromelanin depends on the amount of excess cytosolic dopamine that has not been transferred into synaptic vesicles.[4,5] As a result of paramagnetic properties and magnetization transfer (MT) effects, neuromelanin-iron complexes cause T1-shortening.[6] This creates a notable contrast in NM-MRI signal between the SN and the surrounding brain tissue. Multiple NM-MRI studies have demonstrated elevated neuromelanin concentration in the SN of patients with schizophrenia compared to healthy controls (HC).[7]

The findings of elevated striatal DSC and neuromelanin concentration in the SN of patients with schizophrenia suggest that these measures might relate positively to each other. This is supported by the observation that NM-MRI signal in the SN is positively associated with amphetamine-induced dopamine release (i.e., another indicator of striatal hyperdopaminergia) in the whole striatum, as assessed with [^{11}C]raclopride PET, across patients with schizophrenia and HC.[3] It is unknown though how striatal DSC and NM-MRI signal in the SN, as measures of nigrostriatal functioning, are interrelated.

Therefore, we investigated the association between NM-MRI signal in the SN and DSC in the whole, associative, limbic, and sensorimotor striatum in HC and patients with a schizophrenia spectrum disorder (SSD). In addition, we explored the association between nigral DSC and NM-MRI signal in the SN of patients and HC. We hypothesized that NM-MRI signal in the SN is positively correlated with striatal DSC in both groups. We assessed the relation between NM-MRI signal and striatal DSC in separate groups since meta-analytic evidence shows that both measures are altered in patients with schizophrenia compared to controls,[1,2,7] and more importantly, striatal DSC seems to fluctuate with psychotic symptom severity and medication status in patients,[8-11] whereas there are indications that this is not the case for NM-MRI (unpublished data).

2. Experimental procedures

This study combines data from two patient and two HC cohorts, collected in the context of three Dutch studies approved by the Medical Ethical Committees of Leiden, The Hague, and Delft (NL72218.058.20), Amsterdam UMC, University of Amsterdam (NL63410.018.17), and the East Netherlands (NL72675.091.20). All participants gave written informed consent. PET data of all subjects have not been previously published. NM-MRI data of 9 patients are included in the analysis of another manuscript.[12]

2.1. Participants

For this study, early psychosis patients who recently experienced an episode of psychosis were recruited via two Dutch mental health institutes (details are explained in eMethods 1). All patients were undergoing treatment and were diagnosed with SSD. Diagnoses were confirmed with the semi-structured Comprehensive Assessment of Symptoms and History (CASH) interview.[13] In addition, HC matched for age, gender, smoking status, and educational level were recruited via social media. Patients and HC were both aged between 18-50 years. Exclusion criteria for patients included onset of first psychotic episode longer than five years ago and previous antipsychotic use longer than one year. Additional exclusion criteria are explained in eMethods 1.

2.2. Design and procedures

Participants were assessed on 1 to 3 testing days. The study procedure consisted of: 1) screening for in- and exclusion criteria and completing measures on medication use (eMethods 2) and symptom severity by use of the Positive and Negative Syndrome Scale (PANSS; patients only)[14] and the Beck Depression Inventory (BDI-II)[15,16]; 2) MRI scan including the NM-MRI; and 3) [^{18}F]F-DOPA PET scan. Data collection occurred between 11/03/2019 and 14/09/2022.

2.3. NM-MRI acquisition

All participants were instructed to refrain from alcohol and cannabis 24 h before the MRI scan. MRI images were acquired on a 3T scanner (Phillips, Ingenia Elition X, Best, The Netherlands) with a 32-channel head coil at the Amsterdam UMC, the Netherlands. Structural whole-brain T1-weighted volumetric images were acquired for NM-MRI slice placement. NM-MRI was acquired with a T1-weighted 2D gradient echo sequence with MT pulse (TR = 260 ms; TE = 3.9 ms; 8 slices; FOV = 162 x 199 mm; slice thickness = 2.5 mm; number of signal averages = 2; FA = 40°; MT frequency offset = 1200 Hz; MT duration = 15.6 ms) (details of scan sequences are described in eMethods 3).

2.4. NM-MRI pre-processing

The NM-MRI scans were pre-processed with a Matlab (MathWorks, Natrick, MA) pipeline,[17] which is extensively described in eMethods 4. In short, NM-MRI images were coregistered to the T1-weighted images. Brain-extracted T1-weighted images were spatially normalized to Montreal Neurological Imaging (MNI) space. Next, the coregistered NM-MRI images were spatially normalized to MNI space using the warping parameters that were used for the normalization of the T1-weighted images. Afterwards, the normalized NM-MRI images were smoothed with a 1-mm full-width-at-half-maximum (FWHM) Gaussian kernel. The NM-MRI signal in the SN was calculated as a contrast-to-noise ratio (CNR) with the crus cerebri (CC) as the reference region, using SN and CC template masks (Figure 1). For each participant, the CNR at each voxel *v* in the SN was calculated as the percent NM-MRI signal difference between a given voxel in the SN mask (I_v) and the mode of the signal intensity in the CC (I_{cc}) (Equation 1). The mode (I_{cc}) was calculated from a kernel-smoothing-function fitted to a histogram of the distribution of all voxels in the CC mask.

$$CNR_V = \left\{\frac{[I_V\text{-mode}(I_{CC})]}{\text{mode}(I_{CC})}\right\} * 100 \qquad \textit{(Equation 1)}$$

2.5. PET acquisition

All participants were asked to refrain from alcohol and cannabis 24 hours, eating and drinking (except water) six hours, and smoking three hours before PET imaging. One hour before the PET scan, all participants received 150 mg carbidopa and 400 mg entacapone to block peripheral metabolization of [^{18}F]F-DOPA.[18,19] Before PET acquisition, a low-dose computed tomography (CT) scan of the brain was acquired for attenuation correction purposes. Subsequently, approximately 185 MBq [^{18}F]F-DOPA was administered as a single intravenous bolus injection. Immediately thereafter a 90-minute dynamic PET acquisition started. PET data were acquired on a Siemens PET/CT system (Biograph mCT FlowTrue-V-128) (FOV = 256 x 256 mm; slice thickness = 2 mm; pixel spacing = 1.59 x 1.59 mm) and binned in 25 frames (5 x 1, 3 x 2, 3 x 3, and 14 x 5 minute[s]) (eMethods 5).

2.6. PET pre-processing

Details of the PET pre-processing are described in eMethods 6. In short, participants who moved > 7.5 mm during the data acquisition were excluded from further analyses, as attenuation correction might no longer be reliable. Structural T1-weighted and PET images were co-registered to a single PET frame acquired 7 minutes post-injection. Next, the T1-weighted images were segmented into white matter (WM), grey matter (GM), and cerebral spinal fluid (CSF). The volumes of interest (i.e., striatum and

cerebellum) were generated based on Hammers' maximum probability atlas.[20] Afterwards, Patlak graphical analysis[21] was used to calculate the influx constant k_i^{cer} (min^{-1}; from here on labelled as k_i^{cer}) as a measure of DSC with the GM of the cerebellum as reference region. Linear fitting was conducted on the PET images acquired between 25 and 90 minutes to acquire a whole-brain parametric image (Figure 2A/B). The k_i^{cer} of the GM striatum was extracted from this parametric image.

A standard MNI brain template was warped with a non-linear affine transformation to the subject's MRI. Thereafter, the same transformation matrix was applied to warp the striatal subdivisions (i.e., associative, limbic, and sensorimotor striatum), as defined in the Oxford-GSK-Imanova brain atlas,[22] from MNI to subject space. Subsequently, the GM k_i^{cer} for voxels with ≥90% probability of belonging to the striatal subdivision was extracted from the whole-brain parametric image. k_i^{cer} in the SN was calculated with a similar method (eMethods 6).

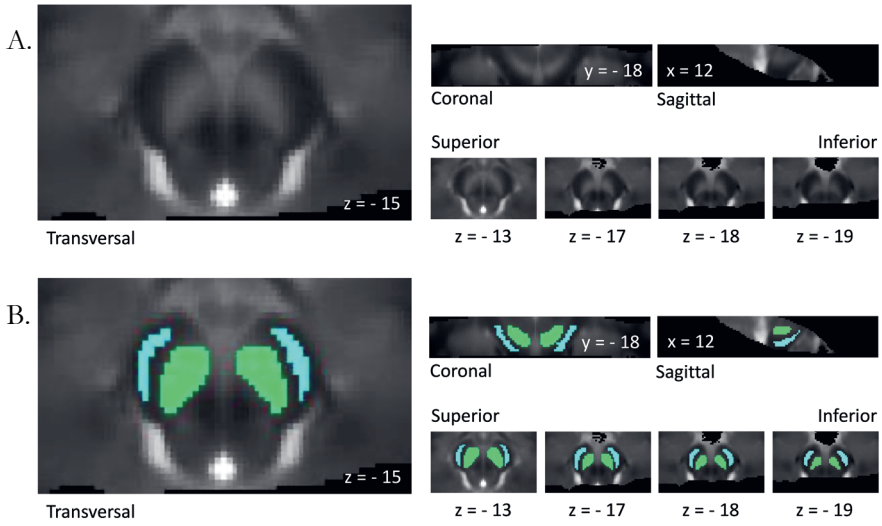


Figure 1. Template masks of the substantia nigra and the crus cerebri. (A) Average image of spatially normalized NM-MRI images from 28 participants included in the primary analyses. The SN is visible as a hyperintense area. (B) Template masks of the SN (in green) and CC (in blue) in MNI space were created by manually tracing the regions on the average NM-MRI image. The template masks were used for calculating the contrast-to-noise ratio in all subjects.
Abbreviations: CC, crus cerebri; MNI, Montreal Neurological Imaging; NM-MRI, neuromelanin-sensitive magnetic resonance imaging; SN, substantia nigra.

2.7. NM-MRI and PET analyses

In line with previous work, our primary analysis consisted of voxelwise analyses conducted in MATLAB.[3,23] We chose voxelwise analyses to reduce statistical circularity in defining the SN region via signal-intensity thresholding and to account for regional heterogeneity of dopamine neurons across tiers without well-defined anatomical

boundaries. Age was used as a covariate in all analyses, as neuromelanin accumulation is known to be age-related.[24] For the primary analysis, we examined the association between striatal DSC and nigral NM-MRI signal in both groups separately using a voxelwise robust linear regression that predicted CNR at every voxel within the SN based on mean k_i^{cer} values (for whole, associative, limbic, and sensorimotor striatum regions of interest [ROIs]) and age (eMethods 8). Significance testing was determined by use of a permutation test in which mean k_i^{cer} values of the striatal ROI were randomly shuffled, 10,000 times, with respect to the individual maps of the NM-MRI signal in the SN. This resulted in a null distribution of the number of SN voxels that exceeded a threshold of $p < 0.050$. The permutation test corrects for multiple comparisons by deciding whether the effect's spatial extent k is larger compared to chance (corrected $p < 0.050$). In case of significant results, we subsequently performed post-hoc partial Spearman's rank-order correlation coefficient tests to address the strength of the correlation (i.e., Spearman's rho). In addition, we calculated the 95%-confidence interval of Spearman's rho by use of the Fisher z-transformation. The associations between the mean CNR values from the significant voxels (thresholded at $p < 0.050$) and mean k_i^{cer} values in the striatal ROI were assessed with age as covariate. We did this with mean CNR values uncorrected and corrected for voxel selection (i.e., obtained by a leave-one-subject-out analysis to get an unbiased effect size). In the leave-one-out analysis significant voxels for each HC were identified in a voxelwise analysis including the complete HC sample except the left-out subject. The significant voxels were used to extract the mean CNR for the left-out subject. We explored the association between nigral DSC and NM-MRI signal in the SN with a similar voxel-based method. Group differences and associations between imaging and clinical variables were assessed as described in eMethods 8.

3. Results

3.1. Participants

Eighteen patients with SSD and 24 HC completed the study. For various reasons, we were unable to use the data of 14 participants (n = 6 patients, n = 8 HC; eResults 2). The final sample included 12 patients and 16 HC. Average head movement during the PET scan was comparable in these groups (patients: 2.39 mm, SD = 1.20; HC: 2.10 mm, SD = 1.20; eMethods 9). There were no between-group differences in sex, age, current nicotine use, ethnicity, educational level, or injected [^{18}F]F-DOPA dose (Table 1). However, patients had significantly higher BDI scores compared to HC ($U = 6.000$, $p < 0.001$). The voxelwise analysis to address group differences in CNR signal in the SN revealed no voxels with significant differences between groups (robust linear regression controlling for age, CNR patients > CNR HC corrected $p = 0.377$, CNR HC > CNR patients corrected $p = 0.760$, permutation test). We found no significant group

differences for mean k_i^{cer} values in the whole, associative, or limbic striatum, or the SN. Patients exhibited lower mean k_i^{cer} values in the sensorimotor striatum than HC (U = 45.000; p = 0.018). This was no longer the case when using GM k_i^{cer} of voxels with ≥60% instead of ≥90% probability of belonging to the sensorimotor striatum (eMethods 7; eResults 3). Non-specific uptake of [^{18}F]F-DOPA in the cerebellum was not significantly different in patients and HC (eFigure 1; eResults 4).

Table 1. Sample characteristics.

	Patients (n = 12)	**HC (n = 16)**	**p-value**
Demographics and clinical characteristics			
Sex (F/M)	2/10	4/12	0.673[a]
Age in years, mean (SD)	20.8 (2.7)	24.5 (6.2)	0.129[b]
Current nicotine use[c] (Yes/No)	4/8	3/16	0.418[a]
Education, No.	-	-	0.125[a]
Secondary vocational education / Senior general secondary education / Pre-university education	9	6	-
Higher professional education / University education (Bachelor's degree)	3	9	-
University education (Master's degree)	0	1	-
Ethnicity, No. (White/Other)	10/2	15/1	0.560[a]
Injected [^{18}F]F-DOPA dose in MBq, mean (SD)	180.6 (13.8)	179.1 (15.5)	0.963[b]
Number of days between [^{18}F]F-DOPA PET and NM-MRI, mean (range)	14.8 (0-33)	5.4 (0-71)	**0.001**[b]
PANSS at study enrollment	-	-	-
Positive score, mean (SD)	12.2 (5.0)	NA	NA
Negative score, mean (SD)	12.9 (6.2)	NA	NA
General score, mean (SD)	25.1 (8.7)	NA	NA
Total score, mean (SD)	50.2 (14.3)	NA	NA
BDI, mean (SD)	12.8 (8.0)	1.8 (2.1)	**<0.001**[b]
Diagnosis, No.	-	-	-
Schizophrenia	5	NA	NA
Schizoaffective disorder	2	NA	NA
Schizophreniform disorder	3	NA	NA
Unspecified schizophrenia spectrum and other psychotic disorder	1	NA	NA
Other specified schizophrenia spectrum and other psychotic disorder	1	NA	NA
Current 100 mg CPZ-equivalent dose in mg, mean (SD)[d]	398.6 (222.0)	NA	NA
Total days on antipsychotic medication, mean (SD)[d]	122.1 (98.0)	NA	NA
CPZ dose-years, mean (SD)[d]	0.078 (0.088)	NA	NA

Table 1. (Continued).

	Patients (n = 12)	HC (n = 16)	p-value
NM-MRI and [^{18}F]F-DOPA PET outcome parameters			
CNR, mean (SD)[e]	15.3 (1.0)	15.0 (1.6)	0.889[b]
k_i^{cer} WS, mean (SD)	0.0159 (0.0024)	0.0164 (0.0011)	0.227[b]
k_i^{cer} LST (0.9 threshold), mean (SD)	0.0173 (0.0021)	0.0179 (0.0012)	0.163[b]
k_i^{cer} AST (0.9 threshold), mean (SD)	0.0189 (0.0026)	0.0194 (0.0014)	0.486[b]
k_i^{cer} SMST (0.9 threshold), mean (SD)	0.0196 (0.0037)	0.0216 (0.0019)	**0.018**[b]
k_i^{cer} SN, mean (SD)	0.0099 (0.0014)	0.0099 (0.0011)	0.329[b]

Abbreviations: AST, associative striatum; BDI, Beck Depression Inventory; CNR, contrast-to-noise ratio; CPZ, chlorpromazine; F, female; HC, healthy controls; LST, limbic striatum; M, male; MBq, megabecquerel; NA, not applicable; NM-MRI, neuromelanin-sensitive magnetic resonance imaging; PANSS, positive and negative symptom scale; PET, positron emission tomography; SD, standard deviation; SN, substantia nigra; SMST, sensorimotor striatum; WS, whole striatum. Significant results are bold.
[a]Group differences were assessed with Fisher's exact test. [b]Group differences were assessed with the Mann-Whitney U test. [c]Current nicotine use is defined as having used nicotine daily for at least one month in the past twelve months. [d]During the first scan. [e]Based on average for the whole SN mask (i.e., not the voxelwise analysis).

3.2. Voxelwise and post-hoc analyses of the relationship between neuromelanin and DSC

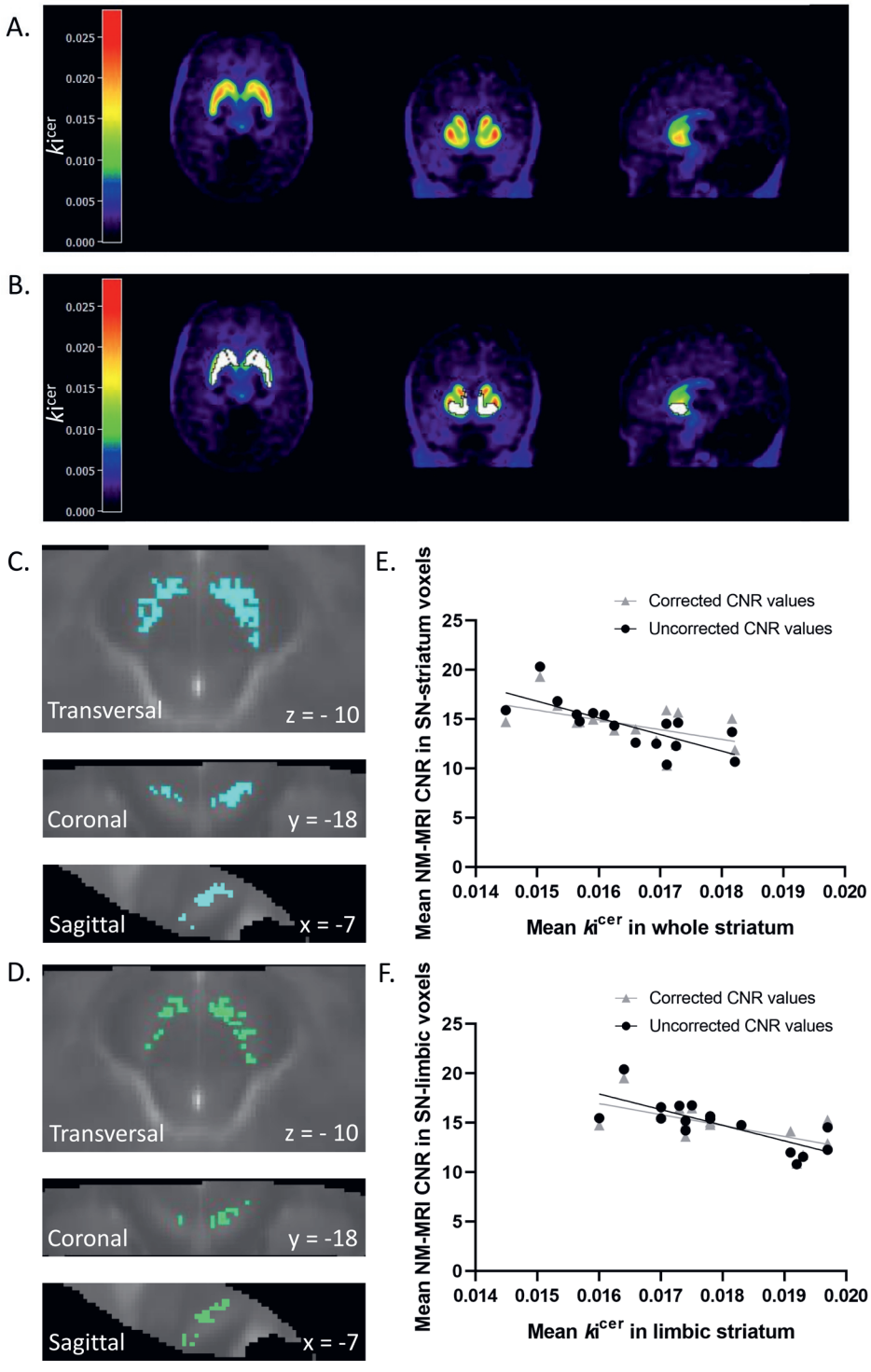
We found a significant negative association in HC between mean k_i^{cer} values in the whole striatum and CNR in a subset of voxels in the SN (hereafter called SN-striatum voxels; 218 of 1,480 voxels at $p < 0.050$, robust linear regression controlling for age; corrected $p = 0.033$, permutation test; peak voxel MNI coordinates [x, y, z]: -5, -12, -9 mm; Figure 2C). Similarly, we found a subset of voxels in the SN of HC (hereafter called SN-limbic voxels) that demonstrated a significant negative association between CNR and mean k_i^{cer} values in the limbic striatum (333 of 1,480 voxels at $p < 0.050$, robust linear regression controlling for age; corrected $p = 0.005$, permutation test; peak voxel MNI coordinates [x, y, z]: -6, -19, -13 mm; Figure 2D). As previous research found a strong correlation between mean striatal k_i^{cer} values for data from a 95-min and 60-min acquisition,[25] we performed a sensitivity analyses in which we repeated the voxelwise analysis for the whole and limbic striatum with four additional HC who were excluded due to movement. We applied linear fitting on the PET images of these four HC acquired between 25 min and the start of substantial (> 7.5 mm) movement. Similarly to the previous findings, we found largely overlapping voxels within the SN where CNR significantly negatively correlated with k_i^{cer} values in the whole and limbic striatum (whole striatum: $p = 0.021$; limbic striatum: $p = 0.015$; eResults 5). We performed an additional sensitivity analysis in which we repeated the voxelwise analysis for the whole and limbic striatum without five HC who fasted for two instead of six hours. This resulted in a borderline significant negative association between mean k_i^{cer} values in the

limbic striatum and CNR in a smaller (compared to the primary analysis) subset of voxels in the SN (p = 0.051; eResults 6). The results for the whole striatum were no longer significant (p = 0.167).

There were no significant associations between CNR in the SN and mean k_i^{cer} values in the associative and sensorimotor striatum in HC and no significant associations between CNR in the SN and mean k_i^{cer} values in any of the striatal ROIs in patients (eResults 7). We repeated the voxelwise analysis in patients for the whole and limbic striatum with three additional patients who were excluded due to movement. This resulted in non-significant findings (eResults 5). The results of the striatal subdivisions did not change when using the mean k_i^{cer} values with ≥60% instead of ≥90% probability of belonging to the striatal subdivision (eResults 8). Our exploratory analyses revealed no subsets of voxels within the SN where CNR correlated significantly with mean k_i^{cer} values in the SN in patients or HC (eResults 9).

The post-hoc analyses revealed a significant negative correlation between the mean CNR in SN-striatum voxels and mean k_i^{cer} values in the whole striatum of HC (controlling for age; uncorrected for voxel selection, rho = -0.853, 95%-CI: (-0.560, -0.956), p < 0.001; corrected for voxel selection, rho = -0.445, 95%-CI: (-0.781, 0.091), p = 0.097; Figure 2E). In addition, we found a negative correlation between the mean CNR in SN-limbic voxels and mean k_i^{cer} values in the limbic striatum of HC (controlling for age; uncorrected for voxel selection, rho = -0.840, 95%-CI: (-0.529, -0.952), p < 0.001; corrected for voxel selection, rho = -0.616, 95%-CI: (-0.125, -0.865), p = 0.015; Figure 2F). For completeness, we also assessed the association between mean CNR within the whole SN mask and mean k_i^{cer} values in the different ROIs with age as covariate (eResults 10). We found no significant associations. Exploratory findings of the relationships between imaging and clinical variables are described in eResults 11-14.

Figure 2. (Right page). Results of voxelwise analysis in the substantia nigra. (A) Average k_i^{cer} in voxels throughout the brain of 16 healthy controls (HC) without and (B) with a mask of the limbic striatum (shown in white). The parametric image of each HC was converted to MNI space for visualization purposes. (C) Map of voxels (shown in blue) in which HC exhibit a negative correlation between NM-MRI contrast-to-noise ratio (CNR) and mean k_i^{cer} values in the whole striatum (WS), i.e., SN-striatum voxels. (D) Map of voxels (shown in green) in which HC exhibit a negative correlation between CNR and mean k_i^{cer} values in the limbic striatum (LST), i.e., SN-limbic voxels. (E) Scatterplot displaying the correlation between mean uncorrected and corrected for voxel selection CNR values in SN-striatum voxels and mean k_i^{cer} value in the WS in HC (uncorrected: rho = -0.853, 95%-confidence interval (CI): (-0.560, -0.956), p < 0.001; corrected: rho = -0.445, 95%-CI: (-0.781, 0.091), p = 0.097). (F) Scatterplot displaying the correlation between mean uncorrected and corrected for voxel selection CNR values in SN-limbic voxels and mean k_i^{cer} value in the LST in HC (uncorrected: rho = -0.840, 95%-CI: (-0.529, -0.952), p < 0.001; corrected: rho = -0.616, 95%-CI: (-0.125, -0.865), p = 0.015).



Caption: left page.

4. Discussion

We used NM-MRI and [18F]F-DOPA PET imaging to investigate the association between NM-MRI signal in the SN and DSC in the striatum and SN of patients with SSD and HC. Contrary to our expectations, we found voxels within the SN of HC where NM-MRI signal correlated negatively with DSC in the whole and/or limbic striatum. The negative associations in the limbic subdivision of the striatum of HC were largely confirmed in post-hoc and sensitivity analyses and not found in patients. Our exploratory analysis did not reveal any significant association between DSC in the SN and NM-MRI signal, which is in line with earlier findings in HC.[26]

Our finding of a negative correlation between NM-MRI and [18F]F-DOPA measures in HC is surprising given that the accumulation of neuromelanin is mostly determined by the amount of excessive cytosolic dopamine.[5] The negative correlation in HC might be explained by functioning of the vesicular monoamine transporter-2 (VMAT-2), which transports cytosolic dopamine into synaptic vesicles. VMAT-2 levels were found to be positively associated with tyrosine hydroxylase levels (i.e., the rate-limiting enzyme for dopamine synthesis, which synthesizes L-DOPA from tyrosine) and negatively associated with neuromelanin pigment in the ventral SN of post-mortem human brains.[27] It might therefore be that dopaminergic neurons in the midbrain of HC with greater amounts of dopamine synthesis have more vesicular storage capacity and consequently less neuromelanin deposition in the SN. This is in line with the finding of Sulzer (2000)[5] who found that neuromelanin synthesis is inhibited by adenoviral-mediated overexpression of VMAT-2. In addition, in rat striata, VMAT-2 functionally and physically interacts with the enzymes tyrosine and aromatic acid decarboxylase (which synthesizes dopamine from L-DOPA),[28] indicating that these components of the dopamine system are directly linked to each other. The negative association in HC suggests that NM-MRI and [18F]F-DOPA PET reflect different components of the dopamine system. This is also supported by the fact that striatal [18F]F-DOPA signal decreases with age,[29] while NM-MRI signal increases with age.[24]

We most consistently found a negative association between NM-MRI and [18F]F-DOPA measures in HC for the limbic striatum. The significant findings in the whole striatum might therefore be driven by the association present in the limbic striatum. The voxels in the SN where NM-MRI signal correlated negatively with DSC in the limbic striatum (Figure 2D) largely overlap with the medial SN, which is found to be connected to the ventral striatum (i.e., the anatomical subregion of the striatum previously classified as belonging to the limbic functional subdivision of the striatum).[30,31] In addition, the medial SN is anatomically adjacent to the ventral tegmental area (VTA),[32] which innervates the nucleus accumbens and ventromedial striatum (i.e., mesolimbic dopaminergic pathway). The reason why the association with the limbic striatum is strongest is still unclear, although it is also known that VMAT-2

levels are lower in the more lateral parts of the ventral SN than in the medial parts of the SN.[27]

The lack of a correlation in patients might be explained by the small sample size. Additionally, striatal DSC might fluctuate more over time in patients compared to HC, as striatal DSC is associated with psychotic symptom severity in patients.[8,9] We did not find significant associations between symptom severity and striatal DSC in our sample, which might be due to the relatively low symptom severity in our patients. Illness severity, duration of illness, and antipsychotic medication might affect striatal DSC,[10,11] whereas no changes in NM-MRI signal have been found after six months of antipsychotic treatment in patients with schizophrenia (unpublished data). This suggests that [^{18}F]F-DOPA PET might be a dynamic measure of DSC (i.e., state-like feature of schizophrenia), while as neuromelanin is a deposit, NM-MRI signal in the SN might reflect more chronic changes in dopamine synthesis (i.e., trait-like feature of schizophrenia). Moreover, in patients, the relationship between striatal DSC and VMAT-2 functioning might be dysfunctional. Although VMAT-2 function is unchanged in the striatum[33] and VMAT-2 binding in the ventral brainstem has been found to be elevated in patients with schizophrenia compared to HC,[34] a post-mortem study found decreased VMAT-2 mRNA levels in the SN of patients with schizophrenia.[35] This might indicate that in a subgroup of patients, increased DSC, which is suggested to be a core feature of the illness,[1] might not be accompanied by an increase in VMAT-2 functioning, which would consequently result in more cytosolic dopamine and thereby more deposition of neuromelanin. Finally, besides elevated striatal [^{18}F]F-DOPA utilization (i.e., the net blood-brain clearance), patients with schizophrenia also demonstrated reduced storage or retention of [^{18}F]-fluorodopamine within synaptic vesicles compared to HC.[36] This would be in line with reduced VMAT-2 functioning in patients. Future studies should address [^{18}F]F-DOPA, VMAT-2, and NM-MRI measures in a large cohort of patients and HC to further elucidate the underlying relationships and their time-courses across the lifespan, while taking into account factors such as illness duration, symptom severity, antipsychotic medication, and seasonal effects.[37]

Previous studies reported elevated striatal DSC in patients with schizophrenia.[1] In contrast, we found a significantly lower mean DSC in the sensorimotor striatum and no differences in the other striatal ROIs in patients compared to HC. These inconsistencies might be due to remission of psychosis in some patients, as lower DSC has been reported in the whole, associative, and sensorimotor striatum of patients in psychotic remission.[38,39] The group difference in the sensorimotor striatum did not remain significant when using GM k_i^{cer} of voxels with ≥60% instead of ≥90% probability of belonging to the sensorimotor striatum. This finding might therefore be an incidental finding. In addition, we found no significant group differences for NM-

MRI signal in the SN. This might be due to the small sample size or heterogeneity, as schizophrenia is a heterogeneous disorder and the existence of multiple subgroups of patients with varying neurobiology has been suggested.[40]

A major strength of this study is that, we are the first to combine NM-MRI and [^{18}F]F-DOPA PET in HC and patients with SSD. However, some limitations have to be taken into account. First, due to the difficulty in recruiting this study population, the sample size of our final sample is limited. To increase our sample size, we aggregated data from three studies with similar selection criteria that used the same NM-MRI and [^{18}F]F-DOPA protocols, except for the length of the fasting time. As [^{18}F]F-DOPA competes with other substrates for transport across the blood-brain barrier, this might have influenced the [^{18}F]F-DOPA PET results. The sensitivity analysis, without five HC that fasted for two instead of six hours, remained borderline significant in the LST and was no longer significant for the whole striatum (eResults 6), which is likely due to a lack of power. Second, [^{18}F]F-DOPA PET measures a combination of cellular processes (i.e., uptake and conversion of [^{18}F]F-DOPA, as well as, storage of [^{18}F]-fluorodopamine). Therefore, additional research needs to investigate which specific aspects of striatal DSC are associated with NM-MRI signal in the SN. This might be done with compartmental modelling in combination with arterial blood sampling during data collection, or by use of other PET tracers, such as 6-[^{18}F]Fluoro-l-m-tyrosine, which is not, unlike DOPA ligands, subject to transport into vesicles and post-release processes.[41] Finally, some participants were regular smokers and/or recreationally used drugs (mainly cannabis). We included these subjects, as a substantial part of patients with SSD uses nicotine and cannabis, and excluding these subject will therefore make recruitment even more difficult and result in a non-representative sample. We found no association between the number of cigarettes or cigars daily smoked by the tobacco users in our sample (4 patients; 2 HC; i.e., during the period when the subject used the most in the 12 months before study participation) and mean CNR in the SN or mean k_i^{cer} values in the whole, associative, limbic, and sensorimotor striatum or SN. Although acute effects of smoking on our imaging measures were likely to be small as the majority of subjects were nonsmokers and others were instructed to refrain from smoking two/three hours before the [^{18}F]F-DOPA PET scan, effects of smoking on striatal DSC are not yet completely understood and studies have reported higher,[42] lower[43] and unchanged striatal DSC in smokers compared to nonsmokers.[44] Further studies are needed to examine the short- and long-term effects of smoking on striatal DSC. Finally, we expect that previous recreational drug use had little effect on our outcome measures, as we selected participants with little to no drug use, who were not dependent on any substance.[45]

5. Conclusions

NM-MRI and [^{18}F]F-DOPA PET are negatively related to each other in HC, but not significantly in patients with SSD. These results indicate that [^{18}F]F-DOPA PET and NM-MRI are measures that reflect different aspects of dopaminergic functioning. We hypothesize that the negative correlation between neuromelanin and striatal DSC in HC might be explained by VMAT-2 functioning. A lack of a correlation in patients might be due to the small sample size or might be explained by effects of symptom severity or antipsychotic medication. In addition, striatal [^{18}F]F-DOPA PET might reflect a dynamic, state-like, aspect of dopaminergic functioning, while NM-MRI signal in the SN might reflect a chronic, trait-like, aspect of dopaminergic functioning. Future studies should assess the interrelationships between DSC, neuromelanin, VMAT-2, and related processes in larger homogeneous cohorts. As NM-MRI is more accessible than PET imaging, this might eventually enable clinicians and researchers to study specific aspects of the dopaminergic system of humans more efficiently and at lower costs.

6. Acknowledgement

We would like to thank all subjects for participating in this study.

7. Author contributions

CvH, MvdP, MH, RvH, LdH, JPS, TvA, JB, and EvdG designed and planned the study. CvH, MvdP, and MH performed the data collection. CvH, CS, and MvdP analyzed the data under the supervision of EvdG, FvV, GH, KW, and MY. CvH wrote the original draft of the manuscript. All authors reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

8. Declarations of interest

All authors declare that they have no conflict of interest.

9. Funding sources

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Appendix to Chapter

Supplementary information can be found in the eBook (pages 185-199):

Chapter

Endocannabinoid levels in plasma and neurotransmitters in the brain: a preliminary report on patients with a psychotic disorder and healthy individuals

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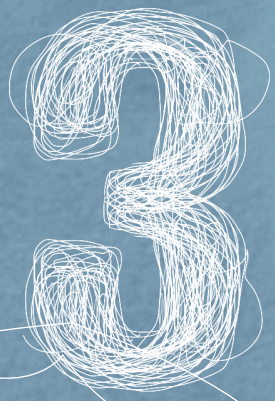
Manuscript submitted (2023)

Part



Towards an individualized approach of disease: Precision psychiatry

Chapter

The association between clinical, sociodemographic, familial, and environmental factors and treatment resistance in schizophrenia: A machine-learning-based approach

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Part

Conclusion

Chapter

Summary

1. Summary of main findings

The overall aim of this dissertation was to advance the current knowledge on neurobiological processes in individuals with an increased risk of developing a psychotic disorder and individuals with non-affective psychotic disorders (NAPD) by using several imaging approaches. By doing so, this work may contribute to the development of a more personalized approach to treatments for psychotic disorders in mental health care. In the first part of this dissertation, neurobiological mechanisms were examined in individuals at increased risk of developing a psychotic disorder, in particular those with 22q11.2 deletion syndrome (22q11DS) (**chapter two – chapter four**). In the second part of this dissertation, neurobiological mechanisms were examined in individuals with NAPD (**chapter five – chapter seven**). To implement personalized treatment approaches in psychosis care, clinicians might use prediction models in the future. These models could utilize, for example, neuroimaging, clinical, and/or sociodemographic data, and support clinical decision-making. In the third part of this dissertation, we elaborate on this future perspective (**chapter eight**). In this chapter, the key findings of this dissertation are summarized.

1.1. Neurobiology of individuals with an increased risk of developing a psychotic disorder: 22q11DS

For more than fifty years, schizophrenia research has mainly been focused on dopaminergic abnormalities.[1] Therefore, in **chapter two**, neuroimaging studies that address several components of the dopaminergic system in individuals at increased risk of developing a psychotic disorder were reviewed. We divided the study cohorts of the reviewed studies into three groups: individuals with a clinical, genetic, or environmental high risk of developing psychosis. The current evidence highlights that striatal dopamine $D_{2/3}$ receptor availability is unaltered in all three high-risk groups compared with healthy individuals. In addition, we found that striatal dopamine synthesis capacity (DSC) was increased in some clinical and genetic high-risk individuals relative to controls (e.g., people that meet clinical criteria for being at ultra-high risk [UHR] of developing psychosis and individuals with 22q11DS), while striatal DSC was decreased in cannabis-using environmental high-risk individuals. It seems therefore likely that individuals with an increased risk of developing psychosis can be stratified into multiple subgroups, with varying risks to develop psychosis and underlying neurobiology. Overall, these findings support the hypothesis that dopaminergic abnormalities already occur in some high-risk individuals before they develop a psychotic disorder. These alterations may facilitate early detection and intervention of psychotic disorders.

As adults with 22q11DS also have an increased risk of early-onset Parkinson's disease[2] and this disease is characterized by the loss of striatal dopamine transporter binding,[3] we aimed to investigate differences in the availability of the striatal dopamine transporter between individuals with 22q11DS, individuals with 22q11.2 duplication

syndrome (22q11DUP), and healthy volunteers in **chapter three**. For this purpose, we set up an [^{123}I]FP-CIT single photon emission computed tomography (SPECT) study in individuals with 22q11DS or 22q11DUP and healthy volunteers. Although we found no statistically significant group differences, individuals with 22q11DS had numerically higher mean striatal [^{123}I]FP-CIT binding ratios than HC, who had numerically higher mean striatal [^{123}I]FP-CIT binding ratios than individuals with 22q11DUP. As we did report some moderate-to-large effect sizes, this suggests that group differences could be verified in a somewhat larger cohort. Future larger studies are necessary to replicate our preliminary findings and investigate whether dopamine transporter imaging could be used as a predictor of progression to Parkinson's disease in individuals with 22q11DS.

Besides investigating whether neurochemical changes occur in the brains of individuals with an increased risk of developing a psychotic disorder, it provides additional insight to know how these neurochemical changes relate to clinical symptomatology in these patients, as well as, how changes in several neurotransmitter systems interrelate. Therefore, in **chapter four**, we explored, using proton magnetic resonance spectroscopy (^{1}H-MRS) and [^{18}F]fallypride positron emission tomography (PET), the relationships between 1) dopamine $D_{2/3}$ receptor availability in the striatum and anterior cingulate cortex (ACC) and 2) glutamate, glutamine, and their combined (Glx) concentrations in the striatum and ACC of individuals with 22q11DS. Additionally, we examined the role of striatal and frontal dopamine $D_{2/3}$ receptor availability in cognitive functioning in 22q11DS, as well as, the association between ACC brain volumes and the concentration of glutamate, glutamine, and Glx in the striatum and ACC of 22q11DS and healthy volunteers. Even though we found no significant associations between frontal or striatal dopamine $D_{2/3}$ receptor availability and glutamate or related metabolite concentrations, our effect sizes were comparable to findings in patients with psychosis. As our sample size was limited, we can therefore not rule out that an association between dopaminergic and glutamatergic functioning does exist in 22q11DS. Moreover, before Bonferroni correction for multiple testing, we found associations in healthy controls between right rostral ACC volume and glutamate concentration in the ACC, between left caudal ACC volume and glutamine concentration in the ACC, and between right caudal ACC volume and Glx concentration in the ACC. No such associations were found in 22q11DS, which suggests that the associations between ACC volumes and glutamate, glutamine, and Glx concentrations in the ACC might differ between groups. Lastly, within the 22q11DS group, the association between dopamine $D_{2/3}$ receptor availability in the left ventral striatum and verbal memory, as well as, the associations between dopamine $D_{2/3}$ receptor availability in the ACC and visual memory, executive functioning, and the composite cognitive score, reached statistical significance. The effect sizes were

noteworthy (i.e., corresponding to strong effects). This suggests that a relationship between dopamine $D_{2/3}$ receptor availability and cognitive functioning might be verified in a larger sample. Although our exploratory study did not reveal a statistically significant association between dopaminergic, glutamatergic, and cognitive functioning, the effect sizes warrant future research on this topic.

1.2. Neurobiology of individuals with a non-affective psychotic disorder

Despite being one of the main origins of dopaminergic input to the striatum, the substantia nigra has been relatively understudied in schizophrenia, in comparison to other brain regions. Hence, in **chapter five**, we presented a literature overview of post-mortem and molecular imaging studies that addressed molecular alterations in the substantia nigra of patients with schizophrenia. We found evidence for hyperdopaminergic functioning in the substantia nigra of patients with schizophrenia (i.e., increased aromatic L-amino acid decarboxylase activity in antipsychotic-free/-naïve patients and elevated neuromelanin accumulation). Reduced γ-aminobutyric acid (GABA)-ergic inhibition (i.e., decreased density of GABAergic synapses, lower vesicular GABA transporter messenger ribonucleic acid [mRNA] levels and lower mRNA levels for $GABA_A$ receptor subunits), excessive glutamatergic excitation (i.e., increased NR1 and Glur5 mRNA levels and a reduced number of astrocytes), and several other disturbances implicating the substantia nigra (i.e., alterations in immune functioning and copper concentrations) could potentially underlie this nigral hyperactivity and associated striatal hyperdopaminergic functioning in schizophrenia. These results highlight the importance of the substantia nigra in the pathology of schizophrenia and suggest that some aspects of molecular functioning in the substantia nigra could potentially be used as treatment targets or biomarkers.

As shown in this dissertation, the dopaminergic system is often investigated *in vivo* by the use of SPECT and PET imaging, such as [^{18}F]F-DOPA PET. However, as these techniques involve exposure to ionizing radiation (which frightens many patients) and can be time-consuming and expensive, we would preferably make use of an alternative method to assess the dopaminergic system, such as neuromelanin-sensitive magnetic resonance imaging (NM-MRI). Before alternative methods can be used in clinical practice, the interrelationships between NM-MRI and PET/SPECT measures should be investigated. Accordingly, in **chapter six**, using NM-MRI and [^{18}F]F-DOPA PET, we investigated the relationship between striatal DSC and neuromelanin in the substantia nigra of patients with NAPD and healthy volunteers. For this purpose, we set up an NM-MRI and [^{18}F]F-DOPA PET study in patients with NAPD and healthy volunteers. In healthy volunteers, we found subsets of voxels within the substantia nigra where NM-MRI signal correlated negatively with DSC in the whole striatum and DSC in the limbic striatum. This was not the case for patients. The negative correlation in

healthy volunteers might be explained by vesicular monoamine transporter-2 (VMAT-2) functioning, while a lack of a correlation in patients might be due to the small sample size, effects of symptom severity or antipsychotic medication. In addition, these findings indicate that [^{18}F]F-DOPA PET and NM-MRI are measures that reflect different aspects of dopaminergic functioning. Striatal [^{18}F]F-DOPA PET might reflect a dynamic, state-like, aspect of dopaminergic functioning, while NM-MRI signal in the substantia nigra might reflect a chronic, trait-like, aspect of dopaminergic functioning. Future studies should assess the interrelationships between DSC, neuromelanin, VMAT-2, and related processes in larger homogeneous cohorts. As NM-MRI is more accessible than PET imaging, this might eventually enable clinicians and researchers to study specific aspects of the dopaminergic system in humans more efficiently and at lower costs. Further research into the exact meaning of the NM-MRI signal is, however, first needed.

Besides neuroimaging markers, other more easily obtainable markers (e.g., blood markers) might be useful to stratify NAPD patients into subgroups. Hence, in **chapter seven**, we assessed differences in plasma concentrations of endocannabinoids between NAPD patients and healthy individuals. Plasma concentrations of N-arachidonoylethanolamine (anandamide) and 2-arachidonoylglycerol (2-AG) were determined by use of liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Contrary to expectation, the plasma concentration of anandamide was significantly lower in patients than in healthy individuals. This did not change after corrections for sex and lifetime cannabis use, and might be explained by the usage of antipsychotic medication that could downregulate anandamide concentrations in blood. We found no group differences with regard to 2-AG plasma concentrations. Additionally, we investigated whether endocannabinoid plasma concentrations were related to dopaminergic, glutamatergic, and GABAergic functioning in both groups, as assessed with [^{18}F]F-DOPA PET and ^{1}H-MRS. We demonstrated a negative association between 2-AG plasma concentration and frontal Glx concentration in patients and a non-significant positive association in healthy individuals. The interaction between group and 2-AG plasma concentration was significantly associated with frontal Glx concentration. Plasma concentrations of 2-AG did not seem to be related to frontal GABA concentrations or striatal DSC in patients or healthy individuals. We also found no compelling evidence for relationships between anandamide plasma concentrations and measures of dopaminergic, glutamatergic, and GABAergic functioning in either group. These preliminary results suggest that peripheral 2-AG might modulate frontal glutamatergic functioning differently in patients with NAPD than in controls. We reported no evidence of a mediating role for peripheral anandamide in regulating neurotransmission. More research in larger cohorts is needed to replicate our findings.

1.3. Towards an individualized approach of disease: Precision psychiatry

Eventually, the obtained information about neurochemical systems in the brains of individuals with an increased risk of developing a psychotic disorder and patients with NAPD might contribute to improved treatment approaches. In addition, the outcome of prediction models, that utilize information about neurochemical processes, might be used as an early indicator of neuropsychiatric disorders or guide treatment choices. Due to the complexity of NAPD, additional information, such as data about clinical and sociodemographic variables, might be necessary to allow for the stratification of patients into subgroups. Therefore, in **chapter eight**, by use of a machine learning model and data from the Genetic Risk and Outcome of Psychosis (GROUP) study, we assessed whether clinical, familial, environmental, and sociodemographic variables, which could potentially predict treatment-resistant schizophrenia (TRS) in the future, were associated with TRS in patients with NAPD. We selected patients who met TRS or antipsychotic-responsive criteria throughout the GROUP study period. The machine learning-based analysis consistently revealed that poor premorbid functioning and younger age at illness onset were important variables that could predict TRS in these patients. In the sensitivity analysis, for which we only selected patients who met the TRS or antipsychotic-responsive criteria at a follow-up assessment but not at the baseline assessment of the GROUP study, we found that poor premorbid functioning and lower educational level were important for the prediction of TRS. Although our machine learning models based on clinical, sociodemographic, familial, and environmental variables only showed a moderate performance in predicting TRS, our findings provide an important base on which precision medicine for TRS can be improved. Future large multi-centre studies are needed to investigate whether the model's performance can be enhanced by adding data from several modalities.

2. References

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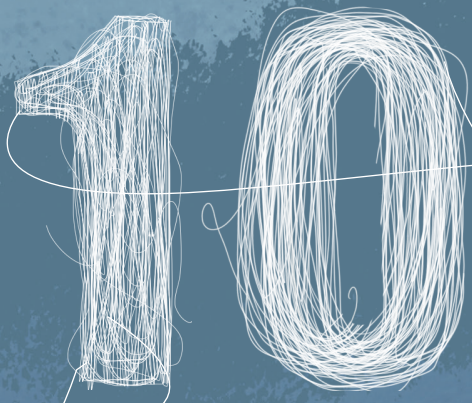
Chapter



General discussion

1. Discussion of main findings

By advancing the current knowledge on neurobiological processes in individuals with an increased risk of developing a psychotic disorder and in individuals with non-affective psychotic disorders (NAPD), this thesis contributes to three important developments: a better understanding of the development of psychotic disorders, the improvement of current and the development of new pharmacological treatments, as well as, the development of personalized treatment approaches for these individuals. In the next section, the key findings of this dissertation and their contribution to these developments will be discussed. Afterwards, the clinical implications of these key findings and suggestions for future research will be described.

1.1. Neurobiological mechanisms underlying an increased risk of developing psychotic disorders

Why do some people develop a psychotic disorder, while others do not? This question has kept many researchers busy for over 60 years. Since then, we have learned that no single factor causes NAPD and that it is likely that many different factors combined, such as drug use, genetic components, and exposure to stress, may give rise to the heterogeneous spectrum of psychotic disorders. Some of these factors might be adjustable and, therefore, prevention strategies targeting these factors could be useful to avert the disorder in some individuals. Accordingly, public health campaigns focused on educating young people about the risks of regular use of high-potency cannabis have been advocated.[1] Also, some specific populations, such as individuals who meet ultra-high risk (UHR) criteria or individuals with 22q11.2 deletion syndrome (22q11DS), have an increased vulnerability to developing a psychotic disorder. Currently, it is unknown which of these individuals will develop a psychotic disorder. It would, therefore, be very useful to understand which factors play a role in the vulnerability and transition to psychosis, so prevention strategies can be tailored to the individuals' needs.

Two leading theories for the pathophysiology of psychosis are the dopamine and glutamate hypotheses.[2-4] Initially, they were proposed separately but integrated they propose that alterations in cortical glutamatergic functioning lead to disruptions in striatal dopaminergic functioning, which then underlies the emergence of positive symptoms. As disruptions in dopaminergic and glutamatergic functioning might already occur in non-psychotic individuals with an increased risk of developing psychosis, **chapters two - four** aimed to provide more insights into these neurochemical systems in high-risk individuals. As neuroimaging studies in patients with NAPD can be confounded by illness-related factors on the brain, such as antipsychotic medication usage[5-7] and alterations in the brain secondary to disease onset, studying neurochemical processes in non-psychotic high-risk groups for psychosis offers a promising approach to increase our understanding of the development of psychotic disorders, which is relevant for individuals with and without NAPD.

In **chapter two**, studies that attempted to identify neurochemical changes in the dopaminergic system in individuals with an increased risk of developing a psychotic disorder were reviewed using a systematic approach. Studies in clinical, genetic, and environmental high-risk groups were taken into account. An important observation was that dopaminergic abnormalities, in particular alterations in striatal dopamine synthesis capacity (DSC), already occur in some high-risk individuals before they develop a psychotic disorder (i.e., individuals who meet UHR criteria [i.e., especially those who will eventually transition to psychosis], individuals with 22q11DS, and cannabis-using individuals). In contrast to our findings, a recent meta-analysis did not find evidence for alterations in striatal presynaptic dopaminergic functioning in clinical or genetic high-risk individuals compared to controls.[8] However, this meta-analysis combined multiple clinical and genetic high-risk subgroups (e.g., individuals with 22q11DS and relatives of patients with schizophrenia were pooled into a genetic high-risk group), as well as, studies that investigated different facets of dopaminergic functioning (e.g., [^{18}F]F-DOPA positron emission tomography [PET] studies that investigated striatal DSC and [^{123}I]IBZM, [^{11}C]-(+)-PHNO, and [^{11}C]raclopride single photon emission computed tomography [SPECT] or PET studies with pharmacological or behavioural challenges that addressed endogenous dopamine release were pooled). This makes it challenging to interpret the outcome of their meta-analysis. In addition, the difference with regard to striatal DSC between clinical high-risk groups and controls was borderline significant ($p = 0.07$).[8] Therefore, it seems possible that high-risk subgroups exist, where dopaminergic alterations occur before the onset of psychosis. This is in line with findings that dysfunctional striatal DSC is associated with the worsening of psychotic symptoms (although not the transition to psychosis) in UHR individuals.[9] Additionally, Stone et al. (2010)[10] reported a trend for an interaction between striatal DSC and glutamate concentration in the hippocampus to predict the transition to psychosis in UHR individuals ($p = 0.07$). These findings suggest that subgroups of high-risk individuals exist with varying risks to develop psychosis and underlying neurobiology. Longitudinal measurements are pivotal to confirm our findings and characterize these subgroups, as well as, the precise trajectories of these neurochemical changes.

Following the systematic review described in **chapter two**, it was noted that some aspects of the dopaminergic system were only investigated in high-risk groups to a limited extent. For instance, the availability of the dopamine transporter was only investigated by two studies focusing on the environmental high-risk group. As individuals with 22q11DS have an increased risk of early-onset Parkinson's disease (PD)[11] and PD is characterized by the loss of striatal dopamine transporter binding,[12] we investigated the availability of the striatal dopamine transporter with [^{123}I]FP-CIT SPECT in individuals with 22q11DS, individuals with 22q11.2 duplication syndrome (22q11DUP), and healthy volunteers in **chapter three**. Although we found no

statistically significant group differences, individuals with 22q11DS had numerically higher mean striatal [^{123}I]FP-CIT binding ratios than HC, who had numerically higher mean striatal [^{123}I]FP-CIT binding ratios than individuals with 22q11DUP. As we did report some moderate-to-large effect sizes, this suggests that group differences could be verified in a somewhat larger cohort.

The findings were in line with our hypothesis, as well as, with previous studies that reported a hyperdopaminergic state in 22q11DS.[13-15] At first, the hyperdopaminergic state in individuals with 22q11DS seems not in accordance with the increased risk of PD, as patients with PD actually demonstrate decreased mean striatal [^{123}I]FP-CIT binding ratios compared to healthy controls.[12] However, this discrepancy can be explained by the auto-toxicity theory. This theory proposes that toxic products of spontaneous and enzymatic oxidation of cytoplasmic catecholamines (e.g., dopamine) affect neuronal integrity and eventually cause death of catecholamine-containing neurons.[16] Therefore, the hyperdopaminergic state might cause neurotoxicity and subsequent death of dopaminergic neurons, which could then explain the increased occurrence of PD in 22q11DS. In accordance with this hypothesis, Butcher et al. (2017)[13] reported that striatal [^{11}C]DTBZ binding, which binds to the vesicular monoamine transporter and therefore gives an indication of the density of dopaminergic neurons, was reduced in a patient with 22q11DS and concomitant PD relative to healthy controls. Future larger studies are necessary to replicate our preliminary findings and investigate whether dopamine transporter imaging could be used as a biomarker to identify and predict PD in individuals with 22q11DS.

As disruptions in striatal dopaminergic functioning in psychosis might be secondary to cortical glutamatergic alterations,[17] we explored the association between these neurotransmitter systems in individuals with 22q11DS in **chapter four**. Due to the small sample size, we were unable to either confirm or firmly invalidate earlier findings of an inverse relation between glutamatergic (i.e., glutamate but not Glx [glutamate plus glutamine] concentration in the anterior cingulate cortex [ACC]) and dopaminergic functioning (i.e., striatal DSC) in patients with psychosis.[18] Our non-significant findings might be explained by methodological differences compared to Jauhar et al. (2018),[18] as we investigated a different aspect of dopaminergic functioning, namely dopamine $D_{2/3}$ receptor availability in the striatum and ACC. As the results described in **chapter two** suggest no alterations in striatal or frontal dopamine $D_{2/3}$ receptor availability and increased striatal DSC in individuals with chromosomal abnormalities (such as 22q11DS), further studies might combine proton magnetic resonance spectroscopy (^{1}H-MRS) imaging with other PET and/or SPECT radiotracers (such as [^{18}F]F-DOPA). Additionally, an inverse correlation between dopaminergic and glutamatergic functioning might be specifically present in high-risk subjects who will eventually transition to psychosis, as Stone et al. (2010)[10] reported a negative association

between glutamate concentration in the hippocampus and striatal DSC in UHR people, which was especially marked in those subjects who went on to develop a psychotic disorder. However, as we did not follow our participants over time, we could not investigate this in our sample. Even though we found no significant associations between frontal or striatal dopamine $D_{2/3}$ receptor availability and glutamate or related metabolite concentrations in the striatum or ACC, our effect sizes were comparable to findings in patients with psychosis.[18] Therefore, we cannot rule out that a significant association between some glutamatergic and dopaminergic markers exists in 22q11DS.

It is important to extend and validate our current research findings and perform longitudinal studies focusing on different aspects of the dopaminergic and glutamatergic systems in larger high-risk samples. In this way, future studies can combine data of homogenous subgroups by use of a quantitative approach. If neurochemical group differences are validated in different cohorts, data on neurochemical processes might also be used as input for practical tools, which can guide clinicians in selecting early prevention strategies.

1.2. Neurobiological mechanisms that underlie symptom severity in high-risk individuals

Besides investigating whether neurochemical changes occur in the brains of individuals with an increased risk of developing a psychotic disorder, it provides additional insight to know how these neurochemical changes relate to clinical symptomatology in these patients. Cognitive impairments typically precede the development of psychosis and are the core determinant of functional disability.[19] In individuals with 22q11DS, cognitive decline is an important indicator of the risk of transitioning to psychosis.[20] The neurotransmitter dopamine plays a role in cognition. A better understanding of how cognitive symptoms in this group relate to dopaminergic functioning as described in **chapters two** and **three** is important, as this might offer the opportunity for the development of new treatments. In **chapter four**, we, therefore, investigated the association between cognitive and dopaminergic functioning in 22q11DS. The negative association between dopamine $D_{2/3}$ receptor availability in the left ventral striatum and verbal memory, as well as, the negative associations between dopamine $D_{2/3}$ receptor availability in the ACC and visual memory, executive functioning, and the composite cognitive score, reached statistical significance. This suggests that it might be possible to verify a relationship between dopamine $D_{2/3}$ receptor availability and cognitive functioning in a larger sample. As a positive, instead of negative, association between striatal dopamine $D_{2/3}$ receptor availability and executive functioning has been reported in healthy individuals, an inverted U-shape-like relation might exist between dopamine $D_{2/3}$ receptor availability and cognitive functioning. Future studies should further investigate this hypothesis, as well as the potential of dopaminergic drugs to reduce cognitive deficits in 22q11DS.

The cognitive deficits observed in 22q11DS might be related to prolonged elevated glutamate levels, as excessive glutamate concentrations are neurotoxic for the brain and can lead to neuronal injury and cell death.[21] Due to the proline dehydrogenase (*PRODH*) haploinsufficiency in 22q11DS, glutamate neuro-excitotoxicity might occur more frequently in this group. This could potentially explain the reduced cortical brain volumes found in individuals with 22q11DS.[22] The relation between glutamatergic and cognitive functioning has been previously reported in the same sample as described in **chapter four**.[23] Verbal and visual memory were negatively associated with glutamate concentration in the ACC of individuals with 22q11DS. However, these associations did not remain significant after correction for multiple testing. In addition to this, we investigated in **chapter four**, the association between ACC brain volumes and glutamatergic functioning in 22q11DS and healthy volunteers. Before correction for multiple testing, we found associations in healthy controls between right rostral ACC volume and glutamate concentration in the ACC, between left caudal ACC volume and glutamine concentration in the ACC, and between right caudal ACC volume and Glx concentration in the ACC. No such associations were found in 22q11DS, which suggests that the associations between ACC volumes and glutamate or related metabolite concentrations in the ACC might differ between groups. Neuro-excitotoxicity due to excessive glutamate concentrations might occur more often in a subgroup of individuals with 22q11DS who transition to psychosis. In line with this, the transition to psychosis has been associated with elevated glutamate levels in the associative striatum.[24] These patients might benefit from drugs that target the glutamatergic system.

1.3. Early prevention strategies for psychosis

Our findings from **chapters two** and **four** suggest that the dopaminergic and glutamatergic systems might not only play a role in the risk of developing a psychotic disorder, but that these systems are also involved in determining symptom severity in (some) individuals with an increased risk of developing a psychotic disorder. Successively, prevention should start very early on in these individuals and medication that targets one or both of these systems might be beneficial to avoid the transition to psychosis or reduce symptoms in some individuals. Accordingly, Latrèche et al. (2022)[25] performed a double-blind randomized controlled clinical trial to investigate the effect of treatment with the antipsychotic risperidone for 12 weeks on psychotic symptomatology in individuals with 22q11DS (n=13). Their initial findings suggest that risperidone might reduce symptoms, mainly negative symptoms. This is surprising as antipsychotic medication antagonise dopamine D_2 receptors, which are expressed predominantly in the striatum, and has limited effectiveness in reducing primary negative symptoms. Their findings might still be relevant, as half of all non-psychotic

individuals with 22q11DS have negative symptoms.[26] However, as the sample size of Latrèche et al. (2022)[25] was very small, their findings might not be generalizable to other patient cohorts and replication in a larger sample is needed.

Moreover, the usage of medication that targets neurochemical systems might not be the ideal strategy for early prevention services, as pharmacological treatment is often accompanied by side effects and the majority of individuals with an increased risk of developing a psychotic disorder will eventually not develop the disorder. Therefore, we suggest future research to focus on 1) developing alternative pharmacological treatments with mild side effect profiles, that target symptoms that are most present in high-risk individuals (i.e., negative and cognitive instead of positive symptoms); 2) improving the selection of individuals for specific prevention strategies, such as exercise training, nutritional supplements, and strategies that focus on reducing drug abuse. Preferably, prevention strategies that are expensive, labour-intensive, and/or associated with potentially hazardous side effects will only be offered to individuals with the highest risk of conversion to psychosis and those who will likely benefit from them, while cheaper, non-invasive, and easily assessable preventions can be offered to a larger group of individuals with a lower conversion risk. In addition, some prevention strategies might also be offered as public health campaigns. In this way, not only a small proportion of help-seeking high-risk individuals can be reached and some prevention strategies, such as exercise training, might be helpful for all youth. Lastly, when providing early interventions their economic and ethical aspects need to be taken into account, as some approaches are costly and identifying someone as at risk might have a negative influence on that person's well-being and unnecessarily exposes these people to the putative harms of stigma.

1.4. Neurobiological mechanisms in psychotic disorders

Chapters five – seven aimed to provide more insights into various neurochemical systems in patients with NAPD. In **chapter five**, post-mortem and molecular imaging studies that investigated molecular alterations in these patients were reviewed with a narrative approach. We focused in particular on alterations in the substantia nigra, as this brain region is relatively understudied in schizophrenia compared to other brain regions. In addition, neuromelanin-sensitive magnetic resonance imaging (NM-MRI) is a recently developed technique, which can be used to non-invasively investigate neuromelanin content in the substantia nigra (e.g., as a proxy for nigrostriatal dopaminergic functioning).[27] Preliminary findings suggest that NM-MRI signal in the substantia nigra is related to response to antipsychotic treatment in NAPD.[28,29] An important finding of **chapter five** was that hyperdopaminergia is not only present in the striatum, as demonstrated by previous research,[30,31] but also in the substantia nigra. As the substantia nigra is one of the main origins of dopaminergic input to the striatum,

molecular alterations in the substantia nigra might underlie the hyperdopaminergic functioning in the striatum. In **chapter five**, we also report evidence for reduced γ-aminobutyric acid (GABA)-ergic inhibition and excessive glutamatergic excitation in the substantia nigra. We hypothesized that these changes could potentially underlie nigral dopaminergic hyperactivity.

Different parts of the striatum and substantia nigra are connected and together these interactions form the striatonigrostriatal (SNS) circuit. Multiple animal studies in macaques and mice have investigated this circuit and implied that within the SNS circuit information can flow via a ventral-to-dorsal[32] and lateral-to-medial route.[33] This occurs via a series of connections between the striatum and substantia nigra (i.e., also referred to as a spiral). The ventral-to-dorsal connections seem to be organized in two ways: via a direct and an indirect loop.[32] From the shell of the striatum connections project to the ventral tegmental area (VTA) and the ventromedial substantia nigra pars compacta (SNc). From the VTA, projections also target the shell of the striatum, forming a direct (i.e., reciprocal) SNS loop. Projections from the SNc do not project back to the shell of the striatum but instead target the core of the striatum. This is called the indirect (i.e., non-reciprocal) SNS loop and forms the first part of the spiral. Successively, from the core of the striatum projections target the SNc (direct loop), as well as, more dorsally located areas in the substantia nigra (indirect loop), thereby continuing the spiral.[32] Haber et al. (2000)[32] proposed that the direct loops consist of projections that directly terminate on a dopaminergic cell (i.e., resulting in inhibition), while the indirect loops consist of projections that indirectly terminate on a dopaminergic cell via a GABAergic interneuron (i.e., resulting in disinhibition). In this way, each part of the spiral can provide inhibitory feedback but also enables the transmission of information to the subsequent step of the spiral via disinhibition. From these findings, the "ascending spiral hypothesis" was proposed, which suggests that the dorsomedial striatum disinhibits dopaminergic signalling in the dorsolateral striatum, via an indirect SNS loop. This hypothesis seems relevant for psychotic disorders, as our findings from **chapter five** suggest that GABAergic functioning in the substantia nigra is reduced. This potentially affects the flow of information through the direct and indirect loops, which results in a dysbalance between inhibition and disinhibition of dopaminergic neurons, and this subsequently alters the functioning of the whole SNS circuit. However, as stated by Ambrosi & Lerner (2022)[34] the ascending spiral hypothesis as described by Haber et al. (2000)[32] is probably not the only way by which regions within the striatum can influence each other. Additional research is therefore needed. Future studies should also address the translatability of the findings of animal studies to SNS circuits in humans.

As the findings of **chapter five** suggest that the substantia nigra might play an important role in the pathology of psychotic disorders, NM-MRI might be used in the

future to investigate the dopaminergic system in the substantia nigra of patients with NAPD. However, the relation between NM-MRI and [^{18}F]F-DOPA PET, which is a commonly used method to investigate the hyperdopaminergic state in NAPD, was yet unknown. In **chapter six**, we, therefore, investigated the interrelationships between nigral NM-MRI and striatal [^{18}F]F-DOPA PET measures in NAPD and healthy individuals. We expected that these two measures would be positively correlated in both groups, as the accumulation of neuromelanin is mostly determined by the amount of excessive dopamine in the cytosol.[35] However, we found voxels within the substantia nigra of healthy individuals where NM-MRI signal correlated negatively with DSC in the whole and/or limbic striatum. This was not found in patients. Our findings indicate that NM-MRI and [^{18}F]F-DOPA PET measures might reflect different aspects of dopaminergic functioning. This is in line with a study in patients with PD, that proposed that [^{123}I]FP-CIT SPECT might be a biomarker for early-stage motor impairments, while NM-MRI might be related to advanced motor symptoms.[36] Nigral NM-MRI signal might thus reflect a chronic, trait-like, aspect of dopaminergic functioning, while striatal [^{18}F]F-DOPA PET might reflect a dynamic, state-like, aspect of this functioning. Vesicular monoamine transporter-2 (VMAT-2) functioning might explain the negative correlation in healthy volunteers. Dopaminergic neurons in the midbrain of healthy volunteers with larger amounts of DSC might have increased VMAT-2 activity and vesicular storage capacity, and consequently less cytosolic dopamine and neuromelanin accumulation in the substantia nigra. As described in **chapter five**, DSC, VMAT-2 functioning, and neuromelanin content might all be altered in the substantia nigra of patients with psychotic disorders and, therefore, these alterations might explain the absence of a correlation in patients. In addition, the lack of a correlation in patients might also be explained by the small sample size, effects of antipsychotic medication, or symptom severity. Future studies should investigate [^{18}F]F-DOPA, VMAT-2, and NM-MRI measures in a large homogeneous cohort of patients and healthy volunteers to further elucidate the underlying relationships, while taking into account factors such as symptom severity, antipsychotic medication, and illness duration. As NM-MRI is more accessible than PET imaging, this might be a technique that researchers and clinicians could use in the future to study specific aspects of the dopaminergic system of humans more efficiently and at lower costs. Further research into the exact meaning of the NM-MRI signal is, however, first needed.

Besides the neurotransmitter systems described so far, other signalling systems, such as the endocannabinoid system, have also been implicated in the pathophysiology of psychotic disorders. For instance, a meta-analysis, across prodromal, first-episode, and multi-episode patients with psychotic disorders, reported increased N-arachidonoylethanolamine (anandamide) levels (i.e., one of two prototypical endogenous endocannabinoids) in the blood of these patients compared to healthy

volunteers.[37] As endocannabinoids can cross the blood-brain barrier,[38] peripheral concentrations of endocannabinoids might be relevant for central brain processes. Hence, in **chapter seven**, we explored whether plasma concentrations of endocannabinoids (i.e., anandamide and 2-arachidonoylglycerol [2-AG]) were related to dopaminergic, glutamatergic, and GABAergic functioning in patients with psychotic disorders and healthy volunteers, as assessed with liquid chromatography coupled to tandem mass spectrometry, [^{18}F]F-DOPA PET, and ^{1}H-MRS. Our preliminary report revealed a significant negative association between Glx concentrations in the ACC (i.e., a proxy for glutamatergic functioning) and 2-AG plasma concentrations in patients, but a non-significant positive association in healthy controls. In line with this, the results of our analyses suggested that the relationship between these two measures differs between the two groups. The negative correlation in patients is expected as binding of 2-AG to the cannabinoid type 1 receptor inhibits the release of glutamate and GABA from presynaptic neurons.[39] Endocannabinoids might, therefore, act as endogenous mediators of neuroprotection to avoid high neurotoxic glutamate concentrations. In controls, we found no evidence for such a protective mechanism. This might be due to the sample size or, as endocannabinoids are released upon demand, this mechanism might only be triggered when needed. Although glutamate, glutamine, and GABA are closely related to each other via the glutamate/GABA-glutamine cycle,[40] 2-AG plasma concentrations were not consistently related to GABA concentrations in the ACC of patients or controls. Striatal DSC was also not associated with 2-AG plasma concentrations in either group. We also found no compelling evidence for relationships between plasma concentrations of anandamide and measures of dopaminergic, glutamatergic, and GABAergic functioning in patients or controls. This might be due to the sample size. Although replication of these findings in larger cohorts is needed, our results might indicate that 2-AG plays a more important role than anandamide in the self-regulatory mechanisms of glutamatergic neurons in the frontal cortex. This would be in line with the observation that 2-AG has been found in the brain at levels 170 times greater than anandamide.[41]

Blood markers, such as endocannabinoid plasma concentrations, might be useful as input for practical tools, which can guide clinicians in selecting intervention strategies for NAPD patients. Hence, in **chapter seven**, we assessed differences in anandamide and 2-AG plasma concentrations between NAPD patients and healthy individuals. Contrary to our expectations, we found lower anandamide plasma concentrations in patients compared to controls. This is not in line with a sensitivity analysis of Minichino et al. (2019),[37] which reported no significant alterations in first-episode patients. Lower anandamide concentrations might be due to antipsychotic medication, as it has been proposed that this type of medication downregulates anandamide levels in blood and all of the patients in our sample used antipsychotics.

Alternatively, lower levels of anandamide might be truly evident in early psychosis patients compared to HC, as this would (theoretically) result in reduced inhibition of several important neurotransmitters in the brain and the subsequent development of psychotic symptoms. It is also possible, however, that anandamide levels are increased in chronically ill patients and that this increase reflects a compensatory mechanism. Additional research is needed to investigate these hypotheses. We found no group differences with regard to 2-AG plasma concentration, which is consistent with findings in patients with schizophrenia and comorbid substance use disorders.[42] Future research is needed to investigate the association between endocannabinoid plasma concentrations and response to pharmacological treatment in NAPD patients.

1.5. Pharmacological treatments for psychotic disorders

Psychotic disorders are very heterogeneous. However, in general, the procedure for treating psychotic disorders is the same for each patient. As a result, one-size-fits-all treatment approaches are imprecise and a relatively large group of 25-33% of all NAPD patients do not respond adequately to the sequential treatment with first- and second-line antipsychotics.[43,44] Moreover, the currently available antipsychotic medication is mainly effective in treating positive symptoms. As the severity of negative and cognitive symptoms is strongly related to functional outcomes,[45] it is important that treatment strategies also comprise these symptom domains. Despite the urgent need to improve the current, and to develop new, pharmacological treatments for psychotic disorders, most drug companies have withdrawn from psychiatric drug development. It is, therefore, no surprise that the US Food and Drug Administration only approved 12 novel drugs in 2011-2021 for psychiatry (of which only a few targeted different mechanisms than the already approved drugs), compared to 135 and 50 novel drugs for oncology and neurology, respectively.[46] If we want to see a change in pharmacological treatment approaches used in psychosis care, more research is needed into neurochemical systems and the connection with symptom severity in individuals with an increased risk of developing a psychotic disorder and patients with NAPD. This is important as drug developers develop their ideas from the clinical and mechanistic understanding of the illness. More insight into neurochemical processes will also allow for the identification of patients who are unlikely to respond to current antipsychotic treatments and increase the efficacy of trials that investigate novel compounds for these patients. Last of all, although some companies have recently been involved in the development of agonists for the serotonin 2A receptor (e.g., psilocybin for depression),[47] more drug companies need to invest in psychiatry again, possibly by the engagement of charitable funders and the government.

1.6. Prediction models for response to antipsychotic treatment

Instead of using a one-size-fits-all treatment approach, in which all patients get treated following the same procedure, it would be preferable if clinicians could use a more personalized treatment approach for treating psychotic disorders, and thereby tailor the treatment procedure to the individual patient. For some patients, this might mean that they start with a current first-line antipsychotic, while other patients might immediately start with the third-line antipsychotic clozapine or alternative interventions. In this way, specific interventions can be offered to those who will likely benefit, while side effects of medication and healthcare costs will be spared for those who will not. One way of implementing a personalized treatment approach into psychosis care might be through the use of prediction models, for example, based on machine learning techniques. These models, based on information provided by the clinician and/or patient, could predict the likelihood that a particular intervention will be effective, and the clinician could then use this information to make patient-specific decisions about intervention strategies. So far, it remains unclear what information can best be used as input for such prediction models. Information about neurochemical processes might be useful for this purpose. However, as NAPD are highly heterogeneous, additional information, such as age at illness onset or sex, might be entered into these models to allow for the accurate stratification of patients into subgroups. To gain more insight into this, **chapter eight** aimed to investigate whether clinical, familial, environmental, and sociodemographic variables, which might be used to predict treatment response in the future, were associated with treatment-resistant schizophrenia (TRS) in patients with NAPD. For this, we utilized a machine learning model and data from the Genetic Risk and Outcome of Psychosis (GROUP) study. Our machine learning-based analysis consistently revealed that poor premorbid functioning and younger age at illness onset could predict TRS in patients with NAPD to a certain extent. In a sensitivity analysis, for which we only selected patients without signs of response or non-response at the start of the GROUP study, we demonstrated that poor premorbid functioning and lower educational level were important for the prediction of TRS. These results are in line with findings from observational studies (as reviewed by Smart et al. (2021)[48]). However, similar to previous studies,[49-52] our machine learning models based on clinical, sociodemographic, familial, and environmental variables showed moderate performance in predicting TRS, with a tendency for a low sensitivity and high specificity. Future large multi-centre studies are necessary to examine whether the model's performance and sensitivity can be enhanced by adding other clinical (i.e., information about early treatment response[53] or psychiatric hospitalization), biological (i.e., peripheral blood markers, such as cytokine,[54] glucose, triglycerides, and alkaline phosphatase levels[55]), or neuroimaging data (i.e., resting-state functional magnetic resonance imaging,[57,58] [18F]F-DOPA PET,[59] and electroencephalography[6]).

1.7. Precision psychiatry in psychosis care

Currently, in the majority of clinical settings, the selection of treatments heavily depends on clinical judgment, in which the clinician combines data utilizing subjective, informal methods. Clinical decisions are dependent on the interplay between, among others, the clinician's educational background, experience with comparable cases, the clinician's knowledge about (inter)national guidelines and empirical literature, as well as, the clinician's ability to integrate and interpret this large amount of complex information.[61] Although previous research has consistently demonstrated that predictions based on statistical methods on average are 10-13% more accurate than predictions based on clinical judgment,[62,63] statistical approaches to guide clinical-decision making are rarely used for prevention/intervention selection in psychosis care.

The findings of **chapter eight** emphasize the potential of prediction models to support clinical decision-making in psychosis care in the future. However, improvements are needed before these models can be implemented. Most importantly, it needs to be clear what information needs to be entered in a prediction model for which outcome and for which population. For example, different information is likely needed to predict treatment response to a first-line antipsychotic compared to the response to clozapine. It is also possible that different information needs to be collected for different populations (i.e., children, adolescents, and adults). With this, the optimal balance between increasing the accuracy of a prediction model and the difficulty to obtain certain data needs to be considered. For instance, the use of neuroimaging is expensive and several of these techniques are not widely available in mental health institutes. In contrast, sociodemographic and clinical information is often already acquired in clinical care or easy, inexpensive, and quick to collect. Although neuroimaging might be more challenging to acquire, Veronese et al. (2021)[59] reported that using a simplified [^{18}F]F-DOPA PET procedure (i.e., only 10 instead of 90 minutes of data acquisition) to guide early initiation of clozapine potentially saves £3400 per patient compared to treatment as usual.

Although a 10-min [^{18}F]F-DOPA PET scan might be well tolerated, this, as well as, the predictive value of [^{18}F]F-DOPA PET, other predictors (e.g., blood markers), and recently developed methods such as NM-MRI, for the prediction of TRS and other outcomes need to be validated in prospective large multi-centre studies. As demonstrated in **chapter eight** and in line with previous findings, our prediction model with clinical, sociodemographic, familial, and environmental variables only showed moderate performance in predicting TRS. Therefore, more research is needed to improve the accuracy and external robustness of prediction models. Additionally, it is important to compare the accuracy of new prediction models for psychosis care to the accuracy of predictions based on clinical judgments. Moreover, the outcomes of prediction models should be interpretable by clinicians. It is important, therefore, that

future studies investigate the utility of prediction models in clinical practice and incorporate requirements on how to use these models from clinicians or other people who will potentially use these models.

2. Clinical implications and suggestions for future research

The studies described in this dissertation were conducted to increase the knowledge about neurochemical systems in patients with NAPD and individuals with an increased risk of developing these disorders. By doing so, these studies aimed to contribute to the development of personalized prevention and intervention strategies. Although there is still a long bumpy road ahead before precision psychiatry can be implemented in psychosis care, several new insights, which could potentially benefit clinical practice and give direction to further research, should be acknowledged. In addition, throughout this general discussion, several suggestions have been put forward for future research. Some additional recommendations should be mentioned.

First, the findings in this dissertation suggest that subgroups of high-risk individuals exist with varying risks to develop psychosis and underlying neurobiology. Successively, prevention strategies could be initiated very early on in these individuals and might be beneficial to avoid the transition to psychosis or reduce symptoms. This, in combination with long waiting times for psychiatric care, emphasizes the importance of primary care providers, such as psychologists affiliated with general practitioners, to recognize subclinical psychotic symptomatology and to subsequently provide patients with corresponding preventions, for example, e-health tools. Some prevention strategies, such as strategies that focus on reducing drug abuse, might be helpful for all youth and might be offered as public health campaigns.

Second, besides conducting brain scans in psychosis care to eliminate organic causes for psychotic disorders, this dissertation proposes that it might be fruitful to utilize neuroimaging for other purposes in the future. For example, if neurochemical alterations in non-responders to non-clozapine antipsychotic medication are confirmed in follow-up research and patients show limited response to these types of medication after the first two weeks of treatment, the clinician might choose not to spend months on going through the entire treatment guideline. Instead, they could have a brain scan made to guide treatment choices about alternative interventions. Future research should investigate which brain scan should be made for which outcome. So far, NM-MRI seems a promising biomarker for investigating dopaminergic (dys)functioning in neuropsychiatric disorders, such as psychotic disorders,[27] PD,[64] cocaine addiction,[65] and major depressive disorder.[66] Fast well-validated NM-MRI sequences might, therefore, be transdiagnostically implemented to guide clinical decision-making in psychiatry.

Third, prediction models can be valuable tools to support clinical decision-making. Some decision tools are already used in Dutch mental health institutes, for

instance, for the identification of patients with major depressive disorder who need highly specialized care.[67] However, statistical approaches to guide clinical-decision making are rarely used for prevention/intervention selection in psychosis care, despite the high impact of treatment non-response on the available treatment capacity of mental health institutes and the advantages that these methods can bring compared to predictions based on clinical judgment. To develop validated prediction models that can be implemented in psychosis care, it is necessary to collect large amounts of data and combine these data in big data repositories. By doing so, the limitation of a small sample, which hampers many neuroimaging studies, can be dissolved. These data repositories can then be used to develop machine learning algorithms that can learn which characteristics are associated with a particular outcome. Subsequently, these algorithms can be used to predict outcomes of prevention and intervention strategies in newly admitted patients. Recently, researchers at King's College London created a data repository with [^{18}F]F-DOPA PET scans, clinical, and sociodemographic information of 597 patients with psychosis and 195 controls.[68] Additional initiatives are required to enable future studies to determine which information should be utilized as input for prediction models and their corresponding outcomes. In this way, the transfer from a one-size-fits-all towards a personalized treatment approach can be made.

Finally, when patients enter a mental health institute a diagnosis is established by the clinician. This is often done by use of the Diagnostic and Statistical Manual of Mental Disorders, version 5 (DSM-5).[69] In the DSM-5 classification system, diagnoses are based on the occurrence of specific symptoms and corresponding criteria. Based on the established diagnosis, a treatment strategy is chosen. However, patients categorized into the same DSM-5 category can present with very different symptoms and respond to medication very differently. This dissertation, therefore, suggests that besides focussing on the type of psychotic disorder (e.g., schizophrenia or schizoaffective disorder), it might be efficient to also categorize patients based on the treatment that they are likely to respond to (e.g., clozapine-responsive schizophrenia). This might enable clinicians to provide patients with effective treatment sooner.

3. Methodological considerations

The strengths and limitations of each study are explained in the corresponding chapter. Nevertheless, some general strengths and limitations of this dissertation should be discussed. First, we combined multiple imaging techniques (i.e., PET, SPECT, NM-MRI, and ^{1}H-MRS) in different cohorts (i.e., high-risk individuals and patients with NAPD) to examine neurochemical changes in psychosis and related disorders. Hereby, we obtained knowledge from different perspectives about high-risk individuals and patients with NAPD, who are in general difficult to recruit for scientific research. A second strength of this dissertation is that it contributed to important collaborations

between different Dutch universities, hospitals, and mental health institutes. Such (international) collaborations are important to advance the field of psychosis research.

A limitation of this dissertation is the relatively small sample size of several studies, due to the complexity of the procedures and the difficulty in recruiting the study populations. Related to this is the challenge of studying neurochemical mechanisms in a population that uses a lot of illicit drugs. As the effects of illicit drug use, as well as, nicotine use on the different neuroimaging outcomes are not yet fully elucidated, we were forced to exclude subjects with extensive substance use. Moreover, we could only invite subjects who were mentally competent to participate in our studies. This implies that subjects understand the purpose of the study, the associated benefit, burden and risks. These restraints might have resulted in a selection bias (i.e., well-functioning patients were selected) and could limit the generalizability of our findings to the whole group of patients with psychotic disorders. In addition, the neuroimaging studies that we performed all had a cross-sectional design, which allows us to only investigate the associations between different concepts, instead of their causal relationships.

4. Concluding remarks

In conclusion, the research described in this dissertation has provided new insights into neurochemical processes in patients with NAPD and individuals with an increased risk of developing these disorders. Information on these processes might be used in the future to guide personalized prevention and intervention strategies for psychosis and related disorders.

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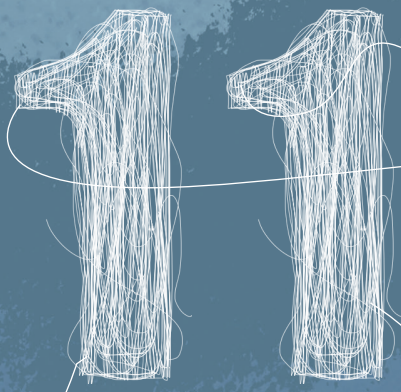
Chapter



Dutch summary | Nederlandse samenvatting

De hobbelige weg van psychotische stoornissen:

Op weg naar gepersonaliseerde behandelingen voor psychotische en aanverwante stoornissen via hersenonderzoek en beeldvormende technieken.

1. Voorwoord

Dit proefschrift is het resultaat van een 4 jaar durende reis, waarin ik onderzoek heb gedaan naar neurobiologische processen in de hersenen van zowel personen met een verhoogd risico op het ontwikkelen van een psychotische stoornis, als personen met een niet-affectieve psychotische stoornis. Het overkoepelende doel van dit proefschrift was om de huidige kennis over neurobiologische processen bij deze twee doelgroepen te vergroten, gebruikmakend van verschillende beeldvormende technieken (zoals positron emissie tomografie [PET] en magnetische resonantie imaging [MRI]). Hiermee draagt dit proefschrift bij aan de ontwikkeling van gepersonaliseerde behandelingen voor psychotische en aanverwante stoornissen binnen de geestelijke gezondheidszorg.

2. Introductie van de onderwerpen in dit proefschrift

Ongeveer 1-3% van de algemene bevolking ontwikkelt gedurende zijn/haar/diens leven een niet-affectieve psychotische stoornis.[1] Niet-affectieve psychotische stoornis is een verzamelterm voor verschillende aandoeningen: waanstoornis, kortdurende psychotische stoornis, schizofreniforme stoornis, schizofrenie, schizoaffectieve stoornis en ongespecificeerde/andere gespecificeerde schizofreniespectrum- of andere psychotische stoornis. Dit zijn ernstige mentale aandoeningen die gekenmerkt worden door het verlies van contact met de werkelijkheid. De meeste patiënten vertonen hallucinaties of wanen, ook wel positieve of psychotische symptomen genoemd, zoals het zien van schimmen of de overtuiging dat je bijvoorbeeld de president van de Verenigde Staten bent. Veel patiënten ervaren daarnaast ook een breed scala aan andere klachten, zoals een gebrek aan motivatie of problemen met informatieverwerking of het werkgeheugen.[2] In tegenstelling tot patiënten met een zogenaamde affectieve psychotische stoornis, staan symptomen die wijzen op een verstoorde stemming – zoals pathologische somberheid of euforie – niet nadrukkelijk op de voorgrond bij patiënten met een niet-affectieve psychotische stoornis.

Verschillende factoren spelen een rol bij het ontstaan van niet-affectieve psychotische stoornissen, zoals middelengebruik, genetische belasting en blootstelling aan traumatische gebeurtenissen. Bepaalde groepen mensen lopen een verhoogd risico op het ontwikkelen van een psychotische stoornis, bijvoorbeeld mensen waarbij psychotische stoornissen voorkomen in de familie en mensen met het 22q11.2 deletiesyndroom (22q11DS). Bij mensen met 22q11DS ontbreekt een deel van chromosoom 22.[3] Kinderen met 22q11DS worden vaak geboren met een hartafwijking of een afwijkend gehemelte.[4] Tevens kunnen andere problemen voorkomen, zoals een

calciumtekort of een ontwikkelingsachterstand.[5] Daarnaast ontwikkelt 20-40% van hen een niet-affectieve psychotische stoornis.[6] Dit percentage is veel hoger dan in de algemene bevolking (1-3%). Momenteel kunnen we niet voorspellen welke individuen met een verhoogd risico deze stoornis daadwerkelijk gaan ontwikkelen. Inzicht krijgen in welke mechanismes in de hersenen een rol spelen bij het wel of niet ontwikkelen van een psychotische stoornis, kan de mogelijkheid bieden om een inschatting te maken van het risico dat een individuele patiënt met 22q11DS loopt op het ontwikkelen van een psychotische stoornis. Aan de hand hiervan kan – indien nodig – vroegtijdig ingrepen worden, waardoor de ziekte mogelijk bij sommigen van hen voorkomen kan worden of het beloop gunstiger kan uitpakken.

Het belangrijkste doel bij de behandeling van niet-affectieve psychotische stoornissen is het verminderen van psychotische symptomen met behulp van antipsychotica, zoals haloperidol en aripiprazol. Deze behandeling is in 25-33% van de gevallen niet effectief,[7] waardoor sommige patiënten langdurig klachten houden en vaak voor lange periodes blootgesteld worden aan medicatie die niet voldoende effectief is. Dit wordt ook wel therapieresistente schizofrenie genoemd. Voor deze patiënten is er een alternatief medicijn beschikbaar, namelijk het antipsychoticum clozapine. Aangezien bij het gebruik van clozapine gevaarlijke (maar zeldzame) bijwerkingen kunnen optreden, hebben patiënten vaak al meerdere andere antipsychotica gebruikt, voordat clozapine wordt voorgeschreven. Behandelaren weten bij het starten van een behandeling niet of een bepaald antipsychoticum zal werken voor zijn/haar/diens patiënt. Zij hanteren daarom vaak een "vallen en opstaan" strategie, waarbij eerst medicijn A geprobeerd wordt en als dit niet werkt er overgestapt wordt naar medicijn B, enzovoort. Voor elke patiënt wordt dezelfde behandelrichtlijn gevolgd. Dit wordt een "one-size-fits-all" behandelstrategie genoemd. Dit is geen ideale strategie, omdat deze ontwikkeld is voor de gemiddelde patiënt. Aangezien de gemiddelde patiënt niet bestaat, betekent dit dat in de praktijk een deel van de patiënten meer of juist minder baat heeft bij het volgen van de richtlijn. We zouden graag, net als in andere medische disciplines zoals oncologie,[8,9] psychotische stoornissen kunnen subtyperen en een behandeling aanbieden die zoveel mogelijk aansluit bij het subtype van de patiënt. Hierdoor wordt de kans op een positieve behandeluitkomst mogelijk het grootst. Het aanbieden van preventie- of interventiestrategieën die aansluiten bij het individu wordt precisiepsychiatrie genoemd. Inzicht krijgen in welke mechanismes in de hersenen een rol spelen bij het wel of niet reageren op antipsychotica, kan de mogelijkheid bieden om een gepersonaliseerde behandeling aan te bieden en de kans op een positieve behandeluitkomst verhogen. Daarnaast zouden vertragingen in het voorschrijven van clozapine en het starten van andere niet-eerstelijnsbehandelingen beperkt kunnen worden.

3. Probleemstelling

Dit proefschrift haakt aan bij twee vragen die vaak gesteld worden aan behandelaren binnen de geestelijke gezondheidszorg: "hoeveel risico loop ik op het ontwikkelen van een psychotische stoornis (bijvoorbeeld in het kader van de aanwezigheid van een genetisch syndroom [zoals 22q11DS], een familiegeschiedenis met psychotische stoornissen of vanwege middelengebruik)?" en "wat is de kans dat antipsychoticum "X" bij mij gaat werken?".

Wanneer behandelaren de antwoorden op deze vragen zouden weten, dan kunnen zij preventie- en interventiestrategieën voor psychotische en aanverwante stoornissen personaliseren. Zij zouden dan specifieke behandelingen kunnen aanbieden aan diegenen die er waarschijnlijk baat bij gaan hebben, terwijl bijwerkingen van medicatie en zorgkosten beperkt kunnen worden voor diegenen die dat hoogstwaarschijnlijk niet gaan hebben. Dit roept echter wel de vraag op: "hoe komen we achter de antwoorden op die twee vragen?"

Het gebruik van een praktisch hulpmiddel (bijvoorbeeld gebaseerd op machine learning technieken) dat de behandelaar ondersteunt en gebruik maakt van verschillende soorten informatie, kan hierbij mogelijk uitkomst bieden. Dit hulpmiddel zou namelijk op basis van informatie verstrekt door de patiënt en/of behandelaar een indicatie kunnen geven van het risico op het ontwikkelen van een psychotische stoornis bij mensen met 22q11DS of de kans dat een specifieke behandeling gaat werken bij mensen met een niet-affectieve psychotische stoornis. Welke informatie nodig is voor het inschatten van iemands risico op een psychotische stoornis of de effectiviteit van antipsychotica is momenteel nog onduidelijk.

Eén mogelijkheid is het gebruiken van informatie over verschillende neurochemische systemen in de hersenen, zoals de dopaminerge, glutamaterge en γ-aminoboterzuur (GABA)-erge systemen. Zo kan men bijvoorbeeld met behulp van beeldvormende technieken (zoals PET en MRI) in verschillende hersengebieden kijken naar de aanmaak van dopamine (i.e., dopaminesynthesecapaciteit) of de beschikbaarheid van receptoren waarop dopamine aangrijpt. Vanwege de complexiteit van psychotische stoornissen is het goed mogelijk dat naast gegevens verkregen met behulp van beeldvormende technieken ook aanvullende informatie nodig is om de juiste inschatting te kunnen maken, zoals gegevens over klinische en sociaal-demografische kenmerken of bepaalde bloedmarkers (bijvoorbeeld plasmaconcentraties van endocannabinoïden). Voordat we gepersonaliseerde preventie- en interventiestrategieën kunnen aanbieden in de klinische praktijk aan individuen met psychotische en aanverwante stoornissen, hebben we meer kennis nodig over neurobiologische processen in de hersenen van deze individuen.

4. Belangrijkste bevindingen van dit proefschrift

4.1. Neurobiologische processen bij mensen met een verhoogd risico op het ontwikkelen van een psychotische stoornis: 22q11DS

In het eerste deel van dit proefschrift hebben we neurobiologische processen onderzocht bij personen met een verhoogd risico op het ontwikkelen van een psychotische stoornis, in het bijzonder personen met 22q11DS (**hoofdstukken twee – vier**). In **hoofdstuk twee** bespreken we de resultaten van 63 eerdere onderzoeken die met behulp van beeldvormende technieken naar verschillende aspecten van het dopaminerge systeem hebben gekeken bij personen met een verhoogd risico op het ontwikkelen van een psychotische stoornis. We verdeelden de deelnemers aan deze onderzoeken in drie risicogroepen en een controlegroep. De eerste risicogroep bestond uit personen met een klinisch hoog risico (zoals individuen met subklinische klachten). De tweede risicogroep bestond uit personen met een genetisch hoog risico (zoals individuen met 22q11DS). De derde risicogroep bestond uit personen met een verhoogd risico door de blootstelling aan omgevingsfactoren die geassocieerd zijn met psychotische stoornissen (zoals individuen die regelmatig cannabis gebruiken). De bestudeerde onderzoeken lieten zien dat de beschikbaarheid van de striatale dopamine $D_{2/3}$-receptor onveranderd is in alle drie de risicogroepen in vergelijking met gezonde individuen. De striatale dopaminesynthesecapaciteit bleek verhoogd te zijn bij sommige individuen met een klinisch of genetisch hoog risico (o.a. individuen met 22q11DS) en verlaagd bij personen met een verhoogd risico vanwege cannabisgebruik. Deze bevindingen suggereren dat personen met een verhoogd risico op het ontwikkelen van een psychotische stoornis verdeeld kunnen worden in meerdere subgroepen. Deze subgroepen lopen waarschijnlijk diverse risico's op het ontwikkelen van een psychotische stoornis en verschillen mogelijk van elkaar in de mate waarin neurobiologische processen afwijken in vergelijking met gezonde individuen. De resultaten van onze systematische review ondersteunen de hypothese dat afwijkingen in het dopaminerge systeem al voorkomen bij sommige personen met een verhoogd risico op het ontwikkelen van een psychotische stoornis, voordat zij daadwerkelijk deze stoornis ontwikkelen. In de toekomst zouden hoog-risico individuen mogelijk geïdentificeerd kunnen worden met behulp van beeldvormende technieken die deze afwijkingen kunnen detecteren. Daarnaast zou informatie over het dopaminerge systeem richting kunnen geven aan welke preventiestrategie het beste ingezet kan worden voor de individuele patiënt.

Uit **hoofdstuk twee** bleek daarnaast dat sommige aspecten van het dopaminerge systeem slechts in beperkte mate onderzocht zijn bij hoog-risico individuen. Zo onderzochten slechts twee studies de beschikbaarheid van de dopaminetransporter. De ziekte van Parkinson wordt gekenmerkt door een afname van de beschikbaarheid van de dopaminetransporter in het striatum.[10] Aangezien personen

met 22q11DS naast een verhoogd risico op het ontwikkelen van een psychotische stoornis, ook een verhoogd risico lopen op het ontwikkelen van de ziekte van Parkinson voor het 50ste levensjaar,[11] onderzochten we daarom de beschikbaarheid van de striatale dopaminetransporter met behulp van [^{123}I]FP-CIT single photon emissie computertomografie (SPECT) bij personen met 22q11DS, personen met het 22q11.2 duplicatiesyndroom (22q11DUP) en gezonde vrijwilligers in **hoofdstuk drie**. Hoewel we geen statistisch significante verschillen vonden tussen de groepen, was de gemiddelde beschikbaarheid van de striatale dopaminetransporter numeriek gezien hoger bij personen met 22q11DS dan bij gezonde vrijwilligers. Gezonde vrijwilligers hadden een numeriek hogere gemiddelde beschikbaarheid van de striatale dopaminetransporter dan personen met 22q11DUP. Aangezien de effectgroottes overeen kwamen met matige tot grote effecten, wijst dit erop dat verschillen tussen de groepen mogelijk vastgesteld kan worden in een grotere groep deelnemers. Aanvullend onderzoek is nodig om onze voorlopige bevindingen te repliceren en te onderzoeken of [^{123}I]FP-CIT SPECT gebruikt kan worden om te voorspellen welke personen met 22q11DS de ziekte van Parkinson gaan ontwikkelen.

Naast het onderzoeken of neurobiologische veranderingen plaatsvinden in de hersenen van mensen met een verhoogd risico op het ontwikkelen van een psychotische stoornis, is het ook belangrijk om te weten hoe veranderingen in verschillende neurochemische systemen met elkaar samenhangen. Eerder onderzoek suggereert namelijk dat afwijkingen in het striatale dopaminerge functioneren bij patiënten met psychotische stoornissen het gevolg zijn van afwijkingen in het glutamaterge functioneren in het frontale deel van de hersenen.[12] Daarom onderzochten we in **hoofdstuk vier** het verband tussen 1) de beschikbaarheid van de dopamine $D_{2/3}$-receptor in het striatum en een frontaal hersengebied (de cortex cingularis anterior [ACC]) en 2) concentraties van verschillende neurometabolieten (i.e., glutamaat, glutamine en hun gezamenlijke concentratie [Glx]) in het striatum en de ACC bij individuen met 22q11DS. Dit deden we met behulp van twee beeldvormende technieken: [^{18}F]fallypride PET en proton magnetische resonantie spectroscopie (^{1}H-MRS). We vonden geen statistisch significante verbanden tussen frontale en striatale beschikbaarheid van de dopamine $D_{2/3}$-receptor en neurometabolietconcentraties bij individuen met 22q11DS. De effectgroottes waren echter wel vergelijkbaar met de effectgrootte van een grotere studie bij patiënten met psychotische stoornissen, waarbij een negatief verband gevonden werd tussen dopaminerge en glutamaterge functioneren.[13] Aangezien er slechts tien personen meededen aan ons onderzoek, kunnen we daarom aan de hand van onze resultaten niet uitsluiten dat er een verband bestaat tussen dopaminerge en glutamaterge functioneren bij 22q11DS.

Voor het ontwikkelen van nieuwe medicijnen voor de behandeling van psychotische en aanverwante stoornissen is het belangrijk om te begrijpen hoe

neurobiologische veranderingen bij mensen met een verhoogd risico op het ontwikkelen van een psychotische stoornis samenhangen met de klachten die deze mensen ervaren. In **hoofdstuk vier** onderzochten we daarom ook of de beschikbaarheid van dopamine $D_{2/3}$-receptoren in het striatum en de ACC samenhing met het cognitief functioneren van de deelnemers met 22q11DS. De associatie tussen de beschikbaarheid van de dopamine $D_{2/3}$-receptor in het ventrale deel van het striatum en verbaal geheugen (i.e., het geheugen van taal in diverse vormen) was niet significant. Dit gold ook voor de associaties tussen de beschikbaarheid van de dopamine $D_{2/3}$-receptor in de ACC en visueel geheugen (i.e., het geheugen van visuele informatie), executief functioneren (e.g., werkgeheugen) en de samengestelde cognitieve score. De effectgroottes kwamen echter wel overeen met sterke effecten. Dit suggereert dat de associaties tussen de beschikbaarheid van de dopamine $D_{2/3}$-receptor en cognitief functioneren bij 22q11DS mogelijk vastgesteld kan worden in een grotere groep deelnemers.

Cognitieve klachten bij mensen met 22q11DS zijn mogelijk het gevolg van verhoogde glutamaatconcentraties in de hersenen.[14] Hoge glutamaatconcentraties kunnen namelijk schadelijk zijn voor de hersenen en ervoor zorgen dat hersencellen afsterven. In **hoofdstuk vier** onderzochten we daarom ten slotte ook of het hersenvolume van de ACC samenhing met de concentratie van glutamaat, glutamine en Glx in het striatum en de ACC bij mensen met 22q11DS en gezonde vrijwilligers. Voor het toepassen van Bonferroni-correctie (om te corrigeren voor het aantal statistische testen dat uitgevoerd wordt) bleek bij gezonde controles het volume van het rostrale deel van de ACC geassocieerd te zijn met de glutamaatconcentratie in de ACC. Tevens hing bij controles het volume van het caudale deel van de ACC samen met glutamineconcentratie en Glx-concentratie in de ACC. Dergelijke associaties werden niet gevonden bij individuen met 22q11DS. Dit suggereert dat de associaties tussen hersenvolume van de ACC en concentraties van verschillende neurometabolieten anders zijn bij gezonde controles dan bij individuen met 22q11DS. De exploratieve bevindingen uit **hoofdstuk vier** benadrukken de noodzaak voor vervolgonderzoek.

4.2. Neurobiologische processen bij mensen met niet-affectieve psychotische stoornissen

In het tweede deel van dit proefschrift zijn neurobiologische processen onderzocht bij personen met een niet-affectieve psychotische stoornis (**hoofdstukken vijf – zeven**). Al meer dan vijftig jaar richt het neurobiologische onderzoek bij psychotische stoornissen zich voornamelijk op het dopaminerge systeem. De substantia nigra, een hersengebied in de hersenstam, is een van de belangrijkste plekken in de hersenen waar dopamine wordt gemaakt. Zenuwcellen vanuit de substantia nigra projecteren naar het striatum en zorgen vervolgens voor de afgifte van dopamine in het striatum. Er wordt gedacht dat hoge concentraties van dopamine in het striatum ten grondslag liggen aan

het ontstaan van psychotische klachten. In **hoofdstuk vijf** bespreken we daarom de resultaten van eerdere postmortale onderzoeken en studies die met behulp van beeldvormende technieken naar moleculaire veranderingen in de substantia nigra hebben gekeken bij patiënten met schizofrenie ten opzichte van gezonde controles. De 37 bestudeerde onderzoeken lieten zien dat het dopaminerge systeem in de substantia nigra overactief is bij patiënten met schizofrenie. Dit uitte zich voornamelijk door middel van een verhoogde activiteit van het enzym aromatisch aminozuur decarboxylase en verhoogde accumulatie van neuromelanine in de substantia nigra. Het overactieve nigrale dopaminerge systeem bij patiënten is mogelijk het gevolg van verminderde GABAerge remming (o.a. door een lagere dichtheid van GABAerge synapsen) en overmatige glutamaterge stimulatie (o.a. door een kleiner aantal astrocyten die glutamaat kunnen opnemen) van dopaminerge zenuwcellen in de substantia nigra. We vonden ook dat veranderingen in het immuunsysteem en afwijkingen qua koperconcentratie in de substantia nigra bij patiënten mogelijk ten grondslag liggen aan de overactiviteit van het dopaminerge systeem in de substantia nigra. Onze narratieve review benadrukt dat de substantia nigra een belangrijke rol speelt in de pathologie van niet-affectieve psychotische stoornissen. Vervolgonderzoek dat zich op dit hersengebied richt is daarom noodzakelijk. Sommige van de gevonden moleculaire afwijkingen zouden mogelijk in de toekomst gebruikt kunnen worden als aangrijpingspunt voor (nieuwe) medicijnen of als biomarker voor bijvoorbeeld het voorspellen van de behandelrespons op antipsychotica.

Het dopaminerge systeem wordt vaak onderzocht bij mensen met behulp van beeldvormende technieken, zoals SPECT en PET. Tijdens deze onderzoeken wordt een kleine hoeveelheid van een radioactieve stof (zoals [^{18}F]F-DOPA) ingebracht in de bloedbaan. Sommige patiënten zijn echter bang voor radioactiviteit. Bovendien zijn dit soort onderzoeken tijdrovend en duur. Aangezien dit het lastig maakt om dergelijke technieken te implementeren in de geestelijke gezondheidszorg, zouden we bij voorkeur het dopaminerge systeem onderzoeken met behulp van alternatieve, toegankelijkere en goedkopere methodes, zoals neuromelanine-gevoelige MRI (NM-MRI). Deze recent ontwikkelde methode meet de concentratie van neuromelanine in de dopaminerge zenuwcellen in de substantia nigra. Neuromelanine is een afbraakproduct van dopamine dat na verloop van tijd ophoopt. NM-MRI wordt nog niet gebruikt in de klinische praktijk, omdat het nog onbekend is hoe het NM-MRI-signaal samenhangt met de metingen van traditionele PET-/SPECT-methodes om naar het dopaminerge systeem te kijken. In **hoofdstuk zes** onderzochten we daarom de relatie tussen striatale dopaminesynthesecapaciteit (gemeten met [^{18}F]F-DOPA PET) en de concentratie van neuromelanine in de substantia nigra (gemeten met NM-MRI) bij patiënten met een niet-affectieve psychotische stoornis en gezonde vrijwilligers. We hadden verwacht een positieve correlatie tussen de twee uitkomstmaten te vinden in beide groepen, maar uit

ons onderzoek bleek echter dat bij gezonde vrijwilligers de neuromelanineconcentratie in de substantia nigra significant negatief gecorreleerd was met de dopaminesynthesecapaciteit in het gehele striatum en het limbische striatum. Bij patiënten vonden we geen statistisch significante correlaties tussen beide uitkomstmaten. De negatieve correlatie in gezonde vrijwilligers kan mogelijk verklaard worden door het functioneren van de vesiculaire monoamine transporter-2 (VMAT-2). Deze transporter zorgt ervoor dat dopamine vanuit het cytosol opgeslagen wordt in synaptische blaasjes, waardoor er minder dopamine omgezet kan worden in neuromelanine. Het ontbreken van een correlatie tussen striatale dopaminesynthesecapaciteit en neuromelanineconcentratie in de substantia nigra bij patiënten heeft mogelijk te maken met de kleine groepsgrootte en/of de invloed van antipsychotica, ziekteduur en ernst van symptomen op de uitkomstmaten. Onze bevindingen suggereren bovendien dat [^{18}F]F-DOPA PET en NM-MRI verschillende aspecten van het dopaminerge functioneren meten. Hierbij weerspiegelt striatale [^{18}F]F-DOPA PET mogelijk een dynamisch aspect van het dopaminerge systeem, terwijl NM-MRI een chronisch aspect weerspiegelt. Vervolgonderzoek dat zich richt op de onderlinge relaties tussen dopaminesynthesecapaciteit, neuromelanineconcentratie, VMAT-2 en andere gerelateerde processen in grotere homogene groepen van patiënten en controles is noodzakelijk. Aangezien NM-MRI toegankelijker is dan SPECT/PET zou dit in de toekomst mogelijk onderzoekers en clinici in staat stellen om het dopaminerge systeem bij mensen efficiënter en tegen lagere kosten te onderzoeken. Vervolgonderzoek naar de exacte betekenis van het NM-MRI-signaal is echter nog wel nodig.

Naast informatie over neurobiologische processen, zou ook informatie over bepaalde bloedmarkers gebruikt kunnen worden als input voor praktische hulpmiddelen, die behandelaren in de toekomst kunnen ondersteunen bij het aanbieden van gepersonaliseerde preventie- en interventiestrategieën voor psychotische en aanverwante stoornissen. Om deze reden hebben we in **hoofdstuk zeven** verschillen in plasmaconcentraties van twee endocannabinoïden, anandamide en 2-arachidonoylglycerol (2-AG), tussen patiënten met een niet-affectieve psychotische stoornis en gezonde individuen onderzocht. De plasmaconcentraties werden bepaald met behulp van vloeistofchromatografie-massaspectrometrie. We hadden verwacht dat anandamide plasmaconcentraties hoger zouden zijn bij patiënten dan bij gezonde individuen, maar ons onderzoek toonde het tegenovergestelde aan. Lagere anandamide plasmaconcentraties bij patiënten kunnen mogelijk verklaard worden door het antipsychoticagebruik van deze deelnemers. We vonden geen verschillen tussen de groepen qua 2-AG plasmaconcentraties. Daarnaast hebben we in **hoofdstuk zeven** in beide groepen gekeken of de plasmaconcentraties van anandamide en 2-AG geassocieerd waren met het functioneren van de dopaminerge, glutamaterge en

GABAerge systemen (gemeten met [^{18}F]F-DOPA PET en ^{1}H-MRS). Frontale Glx-concentratie bleek negatief geassocieerd te zijn met 2-AG plasmaconcentratie bij patiënten. Bij controles vonden we een niet-significante positieve relatie tussen deze twee uitkomstmaten. Ook bleek de interactieterm tussen groep en 2-AG plasmaconcentratie significant geassocieerd te zijn met frontale Glx-concentratie. Dit suggereert dat de relatie tussen frontale Glx-concentratie en 2-AG plasmaconcentratie anders is in beide groepen. Plasmaconcentratie van 2-AG bleek niet consistent gerelateerd te zijn aan frontale GABA-concentratie of striatale DSC bij patiënten of gezonde individuen. Ook vonden we geen overtuigend bewijs voor relaties tussen anandamide plasmaconcentraties en dopaminerge, glutamaterge en GABAerge functioneren in beide groepen. Deze voorlopige resultaten suggereren dat 2-AG in het bloed mogelijk anders geassocieerd is met het frontale glutamaterge systeem bij patiënten dan bij controles. Anandamide lijkt geen rol te spelen bij het reguleren van dopaminerge, glutamaterge en GABAerge neurotransmissie. Vervolgonderzoek is nodig om onze bevindingen te repliceren.

4.3. Op weg naar gepersonaliseerde behandelingen voor psychotische stoornissen: precisiepsychiatrie

In de toekomst zouden clinici praktische hulpmiddelen kunnen gebruiken die, bijvoorbeeld gebruikmakend van informatie over neurobiologische processen, klinische besluitvorming over preventie-/interventiestrategieën kunnen ondersteunen. In het derde deel van dit proefschrift zijn we dieper in gegaan op dit toekomstperspectief (**hoofdstuk acht**). Aangezien mensen met psychotische of aanverwante stoornissen zeer heterogeen zijn in hoe hun klachten zich uiten, is het aannemelijk dat aanvullende informatie (zoals klinische en/of sociodemografische gegevens) nodig is om bijvoorbeeld een indicatie te kunnen geven of een specifieke behandeling wel of niet gaat werken. Om deze reden hebben we met behulp van een machine learning model in **hoofdstuk acht** onderzocht of bepaalde klinische, familiaire en sociodemografische gegevens en gegevens over de leefomgeving van de patiënt geassocieerd zijn met therapieresistente schizofrenie. Dit hebben we gedaan met behulp van data van de Genetic Risk and Outcome of Psychosis (GROUP)-studie. Voor dit onderzoek selecteerden we patiënten die tijdens het eerste onderzoeksmoment of een van de vervolgmetingen na drie en zes jaar voldeden aan criteria voor therapieresistente of antipsychotica-responsieve schizofrenie. Uit de machine learning analyse bleek dat slecht premorbide functioneren van de patiënten en jongere leeftijd bij aanvang van de psychotische stoornis belangrijke voorspellers zijn van therapieresistente schizofrenie. Voor een aanvullende analyse selecteerden we alleen patiënten die bij aanvang van de GROUP-studie nog niet voldeden aan de criteria voor therapieresistente of antipsychotica-responsieve schizofrenie, maar op een later meetmoment wel. Hieruit bleek dat slecht premorbide functioneren en een lager opleidingsniveau belangrijk

waren voor de voorspelling van therapieresistente schizofrenie. De machine learning modellen presteerden echter matig in het voorspellen van therapieresistente schizofrenie. Vervolgonderzoek is daarom nodig om te bepalen hoe de prestatie van het model verbeterd kan worden en welke aanvullende informatie hiervoor nodig is, bijvoorbeeld informatie verkregen via beeldvormende technieken.

5. Implicaties voor de klinische praktijk en toekomstig onderzoek

Het onderzoek beschreven in dit proefschrift is uitgevoerd om de huidige kennis van neurobiologische processen bij personen met een niet-affectieve psychotische stoornis en personen met een verhoogd risico op deze stoornis te vergroten. Dit met het doel om bij te dragen aan de ontwikkeling van gepersonaliseerde behandelingen voor psychotische en aanverwante stoornissen. Hoewel er nog een lange hobbelige weg in het vooruitzicht ligt voordat gepersonaliseerde preventie- en interventiestrategieën voor deze aandoeningen gebruikt kunnen worden in de geestelijke gezondheidszorg, heeft dit proefschrift een aantal belangrijke klinische inzichten opgeleverd. Deze inzichten kunnen mogelijk de klinische praktijk ten goede komen en richting geven aan vervolgonderzoek.

Ten eerste, personen met een verhoogd risico op het ontwikkelen van een psychotische stoornis kunnen mogelijk verdeeld worden in meerdere subgroepen. Deze subgroepen lopen waarschijnlijk diverse risico's op het ontwikkelen van een psychotische stoornis en verschillen mogelijk van elkaar in de mate waarin neurobiologische processen afwijken ten opzichte van gezonde individuen. Het vroegtijdig starten van preventiestrategieën bij (sommige van) deze subgroepen kan mogelijk nuttig zijn om de ziekte te voorkomen of het beloop gunstiger te laten uitpakken. Dit – in combinatie met de lange wachttijden in de geestelijke gezondheidszorg – benadrukt het belang van eerstelijnszorgverleners (zoals psychologen verbonden aan huisartsen) om subklinische psychotische symptomen te herkennen, zodat passende preventiemiddelen (bijvoorbeeld e-health) aangeboden kunnen worden. Voor een deel van deze patiënten is een verwijzing naar de geestelijke gezondheidszorg daarna mogelijk niet meer nodig, wat de hoge werkdruk en het personeelstekort in de geestelijke gezondheidszorg ten goede kan komen. Sommige preventiestrategieën zouden daarnaast nuttig kunnen zijn voor alle jongeren (ongeacht hun gevoeligheid voor psychotische stoornissen) en breder ingezet kunnen worden als onderdeel van gezondheidscampagnes, bijvoorbeeld campagnes gericht op het terugdringen van middelengebruik onder jongeren.

Ten tweede, hersenscans kunnen mogelijk in de toekomst binnen de geestelijke gezondheidszorg niet alleen gebruikt worden voor het uitsluiten van lichamelijke oorzaken van psychiatrische stoornissen (zoals hersentumoren), maar ook ingezet worden voor andere doeleinden. Zo zouden behandelaren er in de toekomst mogelijk

voor kunnen kiezen om een hersenscan te laten maken bij patiënten waarbij na één à twee weken behandeling met eerstelijns antipsychotica geen of weinig verbetering zichtbaar is. Deze scan zou bepaalde neurochemische processen in kaart kunnen brengen. De behandelaar zou vervolgens deze informatie kunnen gebruiken om eerder over te stappen naar een alternatieve behandeling (zoals clozapine) in plaats van het doorlopen van de gehele behandelrichtlijn. Toekomstige onderzoeken zijn nodig om te bepalen welke beeldvormende technieken ingezet kunnen worden voor welke vraagstelling en voor welke doelgroep. Dusver lijkt NM-MRI een veelbelovende techniek om het dopaminerge systeem te onderzoeken bij neuropsychiatrische stoornissen, zoals psychotische stoornissen,[15] de ziekte van Parkinson,[16] cocaïneverslaving[17] en depressieve stoornissen.[18] Goed gevalideerde, korte NM-MRI-scans zouden daarom mogelijk transdiagnostisch geïmplementeerd kunnen worden om klinische besluitvorming in de psychiatrie te begeleiden.

Ten derde, praktische hulpmiddelen kunnen mogelijk waardevol zijn voor het ondersteunen van klinische besluitvorming. Er worden al een aantal praktische hulpmiddelen gebruikt binnen de Nederlandse geestelijke gezondheidszorg, bijvoorbeeld voor het identificeren van patiënten met een depressieve stoornis met een hoogspecialistische zorgvraag.[19] Praktische hulpmiddelen worden echter nog niet gebruikt voor het selecteren van preventie-/interventiestrategieën bij psychotische en aanverwante stoornissen. Dit ondanks de grote impact van een slechte klinische respons op antipsychotica bij een grote groep patiënten op de beschikbare behandelcapaciteit van geestelijke gezondheidszorginstellingen. Om praktische hulpmiddelen te kunnen ontwikkelen is het belangrijk om grote hoeveelheden data te verzamelen en te combineren in databanken. Deze databanken kunnen gebruikt worden om machine learning algoritmen te ontwikkelen die kunnen herkennen welke kenmerken (bijvoorbeeld leeftijd bij aanvang van de psychotische stoornis) geassocieerd zijn met een bepaalde uitkomst (bijvoorbeeld therapieresistente schizofrenie). Deze algoritmen kunnen vervolgens gebruikt worden om bijvoorbeeld de uitkomst van preventie- en interventiestrategieën bij nieuwe patiënten te voorspellen. Onderzoekers van King's College Londen hebben onlangs een databank gemaakt met [^{18}F]F-DOPA PET-scans en klinische en sociodemografische gegevens van 597 patiënten met een psychotische stoornis en 195 controles.[20] Meer van dit soort initiatieven en het combineren van kennis uit verschillende centra zijn nodig, zodat toekomstige studies kunnen bepalen welke informatie nodig is voor welke vraagstelling en voor welke doelgroep. Via deze weg kan een stap gezet worden richting het toepassen van precisiepsychiatrie en kan mogelijk uiteindelijk de omslag van een "one-size-fits-all" naar een gepersonaliseerde behandelaanpak worden gemaakt.

Ten slotte, zodra iemand in zorg komt bij een geestelijke gezondheidszorginstelling wordt er door de behandelaar een diagnose opgesteld. Dit

wordt in Nederland gedaan aan de hand van de Diagnostic and Statistical Manual of Mental Disorders, versie 5 (DSM-5).[21] In dit classificatiesysteem zijn diagnoses gebaseerd op het optreden van specifieke symptomen en bijbehorende criteria. Op basis van de vastgestelde diagnose wordt vervolgens een behandelstrategie gekozen. Patiënten met dezelfde DSM-5-diagnose kunnen echter zeer verschillen in hoe hun klachten zich uiten en verschillend reageren op antipsychotica. In de toekomst zou het daarom mogelijk efficiënter zijn om patiënten ook te categoriseren op basis van de behandeling waarop ze waarschijnlijk zullen reageren (bijvoorbeeld clozapine-responsieve schizofrenie) in plaats van het type psychotische stoornis (schizofrenie of schizoaffectieve stoornis). Dit zou clinici in staat kunnen stellen om patiënten sneller een effectieve behandeling te bieden.

6. Algemene sterke punten en beperkingen

De sterke en zwakke punten van elk onderzoek worden toegelicht in het desbetreffende hoofdstuk in dit proefschrift. Een algemeen sterk punt van dit proefschrift is het gebruik van meerdere beeldvormende technieken (PET, SPECT, NM-MRI en ^{1}H-MRS) en verschillende doelgroepen om neurochemische processen in psychotische en aanverwante stoornissen te onderzoeken. Hierdoor hebben we vanuit verschillende perspectieven kennis verkregen over personen met niet-affectieve psychotische stoornissen en personen met een verhoogd risico op deze stoornissen. Een sterk tweede punt is dat dit proefschrift heeft bijgedragen aan belangrijke samenwerkingen tussen verschillende Nederlandse universiteiten, ziekenhuizen en geestelijke gezondheidszorginstellingen. Dergelijke (internationale) samenwerkingen zijn belangrijk om het psychoseonderzoek vooruit te helpen.

Een belangrijke beperking van dit proefschrift is dat vanwege de complexiteit van de procedures en de moeilijkheid bij het werven van patiënten, de steekproefomvang van meerdere onderzoeken relatief klein is. Dit komt ook doordat veel patiënten met een psychotische stoornis hard- en/of softdrugs gebruiken. Aangezien de effecten van middelengebruik op de hersenen nog onduidelijk zijn, mochten personen met overmatig middelengebruik niet meedoen aan de verschillende onderzoeken. Bovendien konden alleen personen meedoen die wilsbekwaam waren ter zake van het onderzoek. Dit houdt in dat de proefpersoon het doel van het onderzoek en de bijbehorende voordelen, nadelen en risico's moet begrijpen. Deze beperkingen hebben mogelijk gezorgd voor een selectiebias (waarschijnlijk hebben voornamelijk goed functionerende patiënten meegedaan aan de onderzoeken), wat de generaliseerbaarheid van onze bevindingen kan beperken. Tot slot, de onderzoeken die we hebben uitgevoerd zijn allemaal cross-sectionele onderzoeken, oftewel de onderzoeken zijn op één moment uitgevoerd. Hierdoor hebben we alleen associaties

tussen verschillende concepten kunnen onderzoeken en geen causale verbanden of veranderingen over de tijd.

7. Conclusies

Ter afsluiting, het onderzoek beschreven in dit proefschrift heeft nieuwe inzichten opgeleverd over neurochemische processen bij patiënten met niet-affectieve psychotische stoornissen en personen met een verhoogd risico op het ontwikkelen van deze aandoeningen, in het bijzonder diegenen met 22q11DS. Informatie over neurochemische processen, verkregen met behulp van beeldvormende technieken, zou in de toekomst mogelijk gebruikt kunnen worden om klinische besluitvorming over preventie-/interventiestrategieën te ondersteunen. Dit zou kunnen leiden tot de ontwikkeling van gepersonaliseerde behandelingen voor psychose en aanverwante stoornissen.

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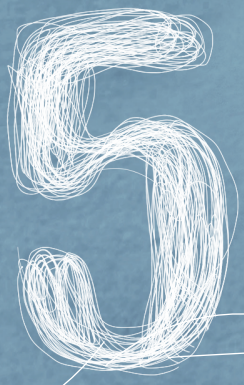

Appendices

Impact paragraph
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1. Impact paragraph

In this chapter, the impact paragraph, the (potential) impact of the research included in this dissertation on science and society will be addressed.

To develop mechanistically novel drugs and eventually provide personalized treatment approaches for psychosis and related disorders, we need a better understanding of the neurochemical systems in the brains of these patients. This dissertation contributed to this need by studying neurobiological processes in individuals with an increased risk of developing a psychotic disorder and individuals with non-affective psychotic disorders (NAPD).

1.1. Implications for science

In terms of scientific impact, this dissertation has several contributions to the existing literature. Our extensive literature review described in **chapter two** is the first to systematically combine neuroimaging studies that addressed the dopaminergic system of multiple high-risk groups for psychotic disorders. We found that striatal dopamine $D_{2/3}$ receptor availability is unaltered in clinical, genetic, and environmental high-risk individuals relative to healthy volunteers. In addition, we found that striatal dopamine synthesis capacity (DSC) was increased in people that meet clinical criteria for being at ultra-high risk of developing psychosis and individuals with 22q11.2 deletion syndrome (22q11DS), while striatal DSC was decreased in cannabis-using environmental high-risk individuals. These findings suggest that neuroimaging techniques might be useful to identify individuals who are likely to transition to NAPD.

In addition, we were one of the first to explore the availability of the striatal dopamine transporter in groups of subjects suffering from 22q11DS and 22q11.2 duplication syndrome (22q11DUP) (**chapter three**). The mean availability of the striatal dopamine transporter was numerically higher in individuals with 22q11DS than in healthy individuals, who had numerically higher availability of the striatal dopamine transporter than individuals with 22q11DUP. This is a contribution to the literature, as only a few molecular neuroimaging studies focused on individuals with 22q11DS and 22q11DUP and our findings confirm the presence of a hyperdopaminergic state in 22q11DS, which has been previously reported by others.[1-3] In addition, we identified the striatal dopamine transporter as a potential target to identify and prevent Parkinson's disease in individuals with 22q11DS.

With the exploratory study described in **chapter four**, we took steps to understand how various aspects of different neurotransmitter systems relate to each other in 22q11DS. These findings may encourage other scientists to investigate this in a larger sample. **Chapter four** also provided preliminary evidence that aspects of dopaminergic and cognitive functioning might be related to each other in 22q11DS.

This is a reason for future research to investigate the potential of dopaminergic drugs to reduce cognitive deficits in 22q11DS.

Our narrative review focusing on molecular aspects of nigral functioning in patients with schizophrenia (**chapter five**) is another add-on to the existing literature. Firstly, because the functioning of the substantia nigra in schizophrenia is relatively understudied relative to the striatum. Secondly, because we found evidence for hyperdopaminergic functioning, reduced γ-aminobutyric acid (GABA)-ergic inhibition, and excessive glutamatergic excitation in the substantia nigra of patients with schizophrenia. These results stimulate critical thinking about and might improve existing theoretical frameworks on the neurobiology of psychotic disorders. In addition, some of the alterations in nigral molecular functioning that we reported have the potential to be used as treatment targets or biomarkers in the future.

In **chapter six**, we combined, for the first time, neuromelanin-sensitive magnetic resonance imaging (NM-MRI) and [^{18}F]F-DOPA positron emission tomography in patients with NAPD and healthy individuals. We expected to find positive associations between these measures in both groups, but instead, we found a negative correlation in healthy individuals and no correlation in patients. From this, it is clear that additional work is needed to understand the meaning of the NM-MRI signal and before this potentially promising method can be used in clinical care to investigate the nigral aspects of the dopaminergic system in neuropsychiatric disorders.

In this dissertation, we also explored the associations between plasma concentrations of two prototypical endocannabinoids and different neurotransmitter systems in patients with NAPD and controls (**chapter seven**). This study is the first of its kind and combines multiple neuroimaging techniques. We found preliminary evidence that 2-arachidonoylglycerol (2-AG) is associated with frontal glutamatergic functioning in patients, but not in controls. It is also the first study worldwide to examine the relationship between striatal DSC and plasma levels of N-arachidonoylethanolamine (anandamide) and 2-AG in patients and controls. Although we found no statistically significant associations between these measures, we took a first step to understand why and how cannabis use might cause the development of psychotic disorders. Eventually, aspects of the endocannabinoid system might be used as drug targets.

Finally, besides investigating neurobiological processes, we also elaborated on the future perspective of moving towards a more personalized approach to treatments of psychotic and related disorders in mental health care (**chapter eight**). We did this by developing a machine learning model to identify clinical, familial, sociodemographic, and environmental variables that could potentially, in the future, predict treatment-resistance in patients with schizophrenia. The model utilized the unique data from the Genetic Risk and Outcome of Psychosis (GROUP) study[4] and showed modest

performance in predicting treatment-resistant schizophrenia. These findings provide future directions for the development of prediction models for psychotic and related disorders. For example, future research needs to investigate whether the model's performance can be improved by adding data from different modalities, as well as, whether prediction models are usable and useful in clinical practice. Essential next steps are also the improvement of the external validation of prediction models and the implementation of these models in real-world settings. Preferably, clinicians should be involved in the development of these practical tools to guide treatment choices in order to bridge the gap between research and clinical practice.

So far, the research included in this dissertation has been well-received in the international academic world. Most results have been published or are currently under review in a peer-reviewed scientific journal, and hence accessible to psychiatric care providers and researchers. Some of the research is published in Open Access journals. Additionally, I have disseminated some of the findings at international scientific conferences (i.e., the Schizophrenia International Research Society conference, the European College of Neuropsychopharmacology [ECNP] conference, and the ECNP neuropsychopharmacology workshop for early career scientists).

1.2. Implications for society

In terms of societal impact, there is still a long way to go. However, I hope that this dissertation will contribute to the development of a more differentiated approach to pharmacological treatments for psychosis and related disorders and eventually the adjustment of treatment guidelines. Moreover, I hope that this dissertation will contribute to the accurate prediction of transition risk to NAPD in high-risk individuals, as well as, treatment response in patients with NAPD. Adjusting the current treatment guidelines and providing personalized treatment options to patients have the following potential long-term impact.

First, it might greatly improve the quality of life of many patients with psychotic or related disorders. During my PhD research, I came in contact with young adults with NADP, 22q11DS, and 22q11DUP. It was devastating to see how, in some cases, their lives were halted due to the symptoms they experienced. This often meant that they could not go to school or work, were isolated from friends and family, and that they could not take care of themselves. In addition, for some of them, it was difficult to find effective medication, which had a judge impact on their quality of life. In general, ineffective treatments result in the discouragement of patients, extended treatment trajectories, chronicity, and high societal costs. Therefore, providing patients with effective treatment sooner might improve these negative consequences and might also be beneficial for the long waiting times for psychiatric care, the high workload for psychiatric care providers, and increase the cost-effectiveness of treatments.

Additionally, on multiple occasions, patients seemed unpleased with the treatment choices that were made by the clinicians. The use of practical tools to guide treatment choices might, therefore, help to improve shared decision-making in the future. For instance, the outcome of these tools might be explained to the patient by the clinician, which facilitates the involvement of patients in this process. This will also make it more understandable for patients why certain treatments are likely to be useful for them, which will make it more appealing to engage in treatment.

As the results of this dissertation may contribute to the future development of personalized treatment approaches for psychotic and related disorders, our findings might not only be relevant to patients but also to psychiatric care providers, health insurance companies, and policymakers. Moreover, our findings might also be relevant to drug companies. Recently, some companies have been involved in the development of agonists for the serotonin 2A receptor.[5] However, more drug companies need to invest in psychiatry, possibly through the engagement of charitable funders and the government. The findings of this dissertation emphasize the importance of such initiatives/collaborations.

Besides working as a researcher, I have also been working as a science editor since the last year of my PhD. In this way, I attempt to communicate research findings to people outside of academia. Some findings of this dissertation have also been presented at local science events organized at a mental health care centre.

In conclusion, the research included in this dissertation is a small, but important step towards a better understanding of neurobiological processes in patients with psychotic and related disorders. This knowledge is needed to develop novel selective drugs, as well as, a more differentiated approach to pharmacological treatments for psychosis and related disorders. Additionally, this knowledge might be useful to accurately predict transition risk to NAPD in high-risk individuals, as well as, the response to antipsychotic treatment in patients with NAPD in the future. Possibly, this will then contribute to the development of personalized prevention and intervention approaches for psychotic and related disorders.

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2. List of publications

2.1. Peer-reviewed journal articles

van Hooijdonk CFM, van der Pluijm M, Smith C, Yaqub M, van Velden FHP, Horga G, Wengler K, Hoven M, van Holst RJ, de Haan L, Selten JP, van Amelsvoort TAMJ, Booij J, van de Giessen E. Striatal dopamine synthesis capacity and neuromelanin in the substantia nigra: A multimodal imaging study in schizophrenia and healthy controls. *Neuroscience Applied 2023;*101134.

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2.2. Submitted work/Work in preparation

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environmental factors and treatment resistance in schizophrenia: a machine-learning-based approach (manuscript under review).

van Hooijdonk CFM, Balvers MGJ, van der Pluijm M, Smith C, de Haan L, Schrantee A, Yaqub M, Witkamp RF, van de Giessen E, van Amelsvoort TAMJ, Booij J, Selten JP. Endocannabinoid levels in plasma and neurotransmitters in the brain: a preliminary report on patients with a psychotic disorder and healthy individuals (manuscript under review).

van Hooijdonk CFM, van Amelsvoort TAMJ, Booij J. Striatal dopamine transporter in individuals with chromosome 22q11.2 copy number variants: an [^{123}I]FP-CIT SPECT study (manuscript in preparation).

Koster M, van der Pluijm M, van de Giessen E, Schrantee A, **van Hooijdonk CFM**, Selten JP, Booij J, de Haan L, Ziermans T, Vermeulen J. The effect of tobacco smoking on metabolite levels in the anterior cingulate cortex of first-episode psychosis patients: A case-control and 6-month follow-up ^{1}H-MRS study (manuscript under review).

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4. Curriculum vitae

Carmen Francina Maria van Hooijdonk was born on 5 September 1995 in Roosendaal en Nispen (Noord-Brabant), The Netherlands. After completing high school at the Norbertuscollege in Roosendaal, she moved to Amstelveen and obtained her bachelor's degree (cum laude) in Medical Natural Sciences at the Vrije Universiteit (VU) Amsterdam in 2016. As part of her bachelor's degree, she studied at the University of Copenhagen for six months via the Erasmus Programme and followed courses on molecular pathology, cellular neuroscience, and neuropharmacology. During this period, she acquired an interest in the human brain. She finished her bachelor's degree with a thesis on the validation of the enzyme phosphodiesterase type 4A as a potential drug target for antischistosomal therapy. Carmen wrote this thesis as part of her internship, under the supervision of dr. Marco Siderius, at the Department of Chemistry and Pharmaceutical Sciences at the VU Amsterdam. Afterwards, she enrolled in the research master's program Cognitive Neuropsychology at the same university in 2016. In her first year, she completed an internship under the supervision of dr. Sara Jahfari and dr. Tomas Knapen at the Department of Experimental and Applied Psychology at the VU Amsterdam, during which she investigated – by use of functional magnetic resonance imaging – how learning from reward can change the attentional profile of the visual cortex. In 2018, Carmen obtained her master's degree (cum laude) after completing an internship at the Donders Institute for Brain, Cognition and Behaviour, at the Radboud University in Nijmegen. As part of this internship, she wrote a thesis on the association between mood and lucid dreaming under the supervision of dr. Martin Dresler. In May 2019, she started as an external PhD candidate at the Department of Psychiatry and Neuropsychology at Maastricht University, for which she was based at the Rivierduinen Mental Health Institute in Leiden. Under the supervision of Prof. dr. Thérèse van Amelsvoort, Prof. dr. Jean-Paul Selten, and Prof. dr. Jan Booij Carmen investigated neurobiological processes, by use of different imaging approaches, in individuals with an increased risk of developing a psychotic disorder and individuals with a non-affective psychotic disorder, which resulted in this dissertation. During the largest part of her PhD project, Carmen worked as a volunteer at the Ronald McDonald House in Leiden. In addition, she acquired an interest in science communication and journalism during her PhD project and started working as a freelance science editor for the Dutch Journal of Psychiatry in August 2022, for which she writes a monthly column on recently published research in the field of psychiatry. Carmen is currently continuing her work as a science editor and, in addition, she is employed as a post-doctoral researcher at the Department of Psychiatry and Neuropsychology at Maastricht University.

The challenges in the treatment of psychotic and related disorders have a lot of similarities with a bumpy road. For 25-33% of all patients with a non-affective psychotic disorder, it is difficult to find effective medication. This results in extended treatment trajectories, the discouragement of patients, and high societal costs. Therefore, providing patients with effective treatment sooner might improve these negative consequences and might also be beneficial for the long waiting times for psychiatric care, the high workload for psychiatric care providers, and increase the cost-effectiveness of treatments.

But how can we improve the treatment of psychotic and related disorders? One possibility might be through the use of prediction models. These models could, based on information provided by the clinician and/or patient, predict the likelihood that a particular intervention will be effective. The clinician could then use this information to make patient-specific decisions about intervention strategies. So far, it remains unclear what information can best be used as input for such prediction models. Information about neurochemical processes might be useful for this purpose.

This dissertation, therefore, explores different neurobiological processes in individuals with psychotic disorders, as well as, individuals with an increased risk of developing these disorders, in particular those with 22q11.2 deletion syndrome.