Psychosis

Psychosis is a serious, debilitating condition and it has been fascinating people for centuries. Its most outstanding features are hallucinations and delusions, the so-called positive symptoms. Other core symptoms of schizophrenia are negative symptoms (impaired motivation, reduction in spontaneous speech, social withdrawal) and cognitive impairment [1,2]. Positive symptoms tend to relapse and remit and can be treated quite effectively with antipsychotics. In contrast, negative symptoms and cognitive impairment tend to be chronic, do not respond well to pharmacological treatment, and impact severely on an individual’s long-term social functioning.

Schizophrenia remains a syndromic concept and is defined as such by DSM-5 criteria. Life time prevalence of schizophrenia is 0.7% [3]. Symptoms typically emerge in adolescence or early adulthood and are often preceded by a prodromal phase or ‘at risk mental state’[4,5]. Of patients diagnosed with schizophrenia, over 50% have intermittent but chronic psychiatric problems, while 20% have chronic symptoms and disability [6]. Unemployment is staggeringly high at 80-90% [7,8]. Life expectancy is reduced by 10-20 years [9]. Schizophrenia is thought to represent the most severe end of a continual range of psychosis liability. At the less severe end of the spectrum lie transient and attenuated psychotic experiences without clinical impact; these are reported to occur in 8% of the general population [10]. Furthermore, there is a large overlap with other psychiatric diagnoses, both in clinical presentation and pathophysiology [1].

Psychosis is a multifactorial condition arising from an interaction between genetic susceptibility and exposure to environmental risk factors [11]. At the beginning of the 21st century, with the field of genetics developing rapidly, expectations were high that the pathophysiology of psychosis would soon be unravelled. Indeed, genome-wide association studies (GWAS) have identified over 100 loci that are associated with psychosis [12,13]. Genes involved in dopamine synthesis, calcium channel regulation, immunity, and glutamate neuroreceptors have been implicated. Furthermore, GWAS data and genotyping allows polygenic risk scores to be calculated for individuals [14]. However, only a minority of the variance of psychosis susceptibility in the population can so far be explained by genetic variants and we are far from understanding the pathophysiology of psychosis. Although much progress has been made, the initial, unrealistically high expectations of genetics have not been met. This has fuelled interest in two existing lines of research: (1) into the social environment as a determinant of psychosis, and (2) into the role of immune dysregulation in the development of psychosis.

The social environment

There is renewed and growing interest among schizophrenia researchers into the role of the social environment as a determinant of psychosis. Epidemiological studies established that environmental factors such as urban place of birth, population density, ethnic minority status, and neighbourhood ethnic density are risk factors for psychosis [11,15–17]. Childhood trauma is also highly prevalent among psychosis patients [18]. The term childhood trauma includes a wide range of adverse experience during early life, including abuse (sexual, physical or emotional), neglect (emotional or physical), peer victimization (bullying), parental loss or separation, and exposure to war or natural disaster. A meta-analysis reported that childhood adversity and trauma increased the risk of psychosis with an odds ratio of 2.8 [18]. Exposure to social stress has been suggested as a common mechanism underlying these associations [19–21]. There is also evidence for a dose-response relationship between social stressors and psychotic disorders, likely reflecting a causal mechanism [22]. Exposure to social stress appears particularly detrimental during early life, while psychosis onset is typically seen in adolescence and early adulthood [11]. It was hypothesized that previous and repeated exposure to social stress leads to stress sensitization, i.e. an increased response to small social stressors in daily life, with cumulative effects resulting in lasting susceptibility in emotional and psychotic reactivity [23]. Genetic factors are assumed to operate by making individuals selectively vulnerable for cumulative exposure to adversity during developmentally sensitive periods. While this model of stress vulnerability and stress sensitization has high face validity, its essentially interactional nature makes it difficult to gather evidence for the model in support of causal relationships or to examine underlying biological pathways [21]. Recall bias and genetic confounding, e.g. the possibility that genetic susceptibility may predispose to environmental exposures, is difficult to exclude. Furthermore, the social environment as a stressor is extremely complex and strongly influenced by an individual’s behaviour; it is difficult to measure or control. Thus, new methods were needed to enable proper examination of possible underlying mechanisms.

Virtual Reality models

Using virtual reality (VR), we designed an interactive three-dimensional (3D) virtual world containing social stress paradigms of high population density, ethnic minority status, and hostile social environments, which was able to induce paranoid thoughts [24]. VR was shown to be a safe method, feasible to execute, and able to elicit persecutory thoughts in individuals with paranoia [25]. It enables individuals with different genetic susceptibilities for psychosis to be randomly allocated to experiments with controlled social risk environments, thus circumventing problems of gene-environment correlation, reverse causation, and recall bias.
Chapter 1

Immune dysregulation

A second line of research has focused on the immune system in psychosis, initially sparked by several findings [26–28]. Firstly, there are striking parallels in the course of autoimmune disorders and psychotic disorders. Both have an age of onset in late adolescence/early adulthood and a relapsing/remitting course of illness, influenced by environmental triggers. There is epidemiological evidence for shared pathways between psychosis and autoimmune disorders. A history of an autoimmune disease was shown to be associated with an increased risk for schizophrenia [29], while autoimmune diseases are more prevalent among individuals with a personal or family history of psychosis [30]. Meta-analysis showed a small effect size of autoimmune disorders and psychosis associations with considerable heterogeneity, but it was consistent across study designs and psychiatric outcomes [31]. Secondly, season of birth has long been reported as a risk factor for schizophrenia. Several epidemiological studies have suggested that exposure to influenza or toxoplasmosis during pregnancy and around birth may explain this seasonal effect [32], although the most recent meta-analysis concluded there was insufficient evidence for maternal influenza to be considered a risk factor for psychosis [33]. Animal studies of maternal immune activation do support the hypothesis that maternal exposure to infectious- or immune activation agents can cause lasting changes in behaviour [34]. GWAS of schizophrenia also support a role for the immune system in psychosis, pinpointing many loci with important roles in the immune system, including the major histocompatibility complex (MHC) [12].

Interest in immunopathological pathways in psychosis was further propagated by the discovery of cases of encephalitis caused by anti-N-methyl-D-aspartate (NMDA) receptor antibodies; some cases had an initial presentation of psychosis [35,36]. It was hypothesized that antibodies perhaps played a much greater role in psychosis than recognized thus far. Anti-NMDA receptor auto-antibodies were found in a small proportion of patients with a psychotic disorder [37], although the clinical significance of this is unclear, especially as one study found equally high prevalences of these antibodies in (healthy) controls as in psychosis patients [38]. It therefore seems unlikely that auto-antibodies have a meaningful role in the majority of psychosis cases.

However, when groups of psychosis patients are compared to groups of healthy controls, there is evidence of a more subtle dysregulation of the immune system. This includes aberrant levels of peripheral cytokines, numbers and functioning of immune cell populations, and activation of microglia in psychosis patients [26]. Immune dysregulation has an impact on brain function, which could make the brain vulnerable to psychosis [39]. The immune system itself is intrinsically sensitive to environmental triggers, including immunological challenges, such as peri-natal or other infections. Psychosocial stressors are also known to modulate the immune system [40].

Research aims and outline of this thesis

I have examined the connections between these two research lines in psychosis (see above) by studying the associations between social stress (sensitization), immune dysregulation, and psychosis susceptibility. I used virtual reality experiments to study the determinants of social stressors in groups with different susceptibilities to psychosis and their responses.

Chapters 2 to 5 all report on the same virtual reality study. The set-up is described briefly in the individual chapters. Each chapter has a different emphasis and a complete overview of the study design is provided in Appendix 1. A concise overview of the players in the immune system is provided in Appendix 2 for readers unfamiliar with immunological terms and concepts.

In Chapter 2 we examined the autonomic stress response of participants with different psychosis susceptibility during exposure to virtual reality social stressors by measuring their heart rate variability and skin conductance levels.

In Chapter 3 we examined the role of childhood trauma in participants with low or high psychosis susceptibility for paranoia or distress in response to social stress.

In Chapter 4 we examined the effects of psychosis susceptibility and childhood trauma on serum levels of inflammatory markers and growth factors.

In Chapter 5 we report on immune cell populations in relation to psychosis susceptibility, childhood trauma, and subjective stress during exposure to virtual reality stressors.

Chapters 4 and 5 are placed in a broader perspective in Chapter 6, in which we systematically review studies on associations between psychosis risk factors like childhood trauma, ethnicity and urbanicity with inflammatory markers in psychosis patients.

In Chapter 7 there is a discussion of our results in the broader perspective of results from meta-analyses and other studies.
Appendix 1. Design of virtual reality experiments

Virtual reality environment
We used virtual reality (VR) to expose participants to social stress in an experimentally controlled manner. The virtual environment was a café or bar with an indoor and an outdoor part, designed by CleVR B.V. (Delft, the Netherlands). In the café, virtual humans (avatars) were sitting or standing at tables, chatting and having drinks (Figure 1.1B) and normal café background noises were played. Participants wore a VR headset, which allowed them to turn around 360 degrees and look around in the virtual café (Figure 1.1A). For walking around, they used a gamepad. Participants were given a simple assignment to ensure they explored the VR environment and approached avatars. Five avatars had a number on their clothing and participants were instructed to explore the café and find these avatars and to report the highest number seen, as well as the sex of the avatar wearing the highest number.

Several variables in the VR environments could be manipulated to create more or less virtual social stress. Firstly, the number of avatars could be varied, to make the café either quieter or more crowded. In the low stress condition, there were six avatars present; in the high stress condition, there were 40. With 40 avatars, the café appeared crowded and the level of perceptual stimuli was higher. This simulated the psychosis risk factor of high population density in urban environments. Secondly, the ethnicity of avatars could be varied to appear either (white) Dutch or (darker-skin) North African. In the low stress condition, most (>80%) of the avatars in the café had an ethnic appearance which best resembled the participant’s ethnicity, i.e. the (own) ethnic density was high. In the high stress condition, most (>80%) of the avatars had an ethnic appearance different from the participant’s, i.e. the (own) ethnic density was low. Thirdly, the facial expressions of the avatars could be varied from neutral to hostile. In the low stress condition, avatars had a neutral facial expression. When participants approached them, the avatars looked at them briefly and then resumed their activities. In the high stress condition, avatars had angry, hostile expressions. The avatars stared at the participants when they approached and at other, random, moments.

Study design
The set-up of our VR study is outlined in Figure 1.2. At baseline (T0), heart rate and skin conductance level were measured during 4 minutes. Subsequently, a blood sample was taken. Next, the participants were asked to complete self-report online questionnaires on:

1. Sociodemographic characteristics including sex, age, ethnicity and education level;
2. Medical history, height, weight, use of psychotropic and other medication (including over-the-counter), substance use (smoking, alcohol, cannabis/THC, and illicit drugs);
3. Childhood trauma (The Childhood Trauma Questionnaire (CTQ));
4. Symptoms of paranoia (Green Paranoid Thoughts Scale (GPTS)), social anxiety (Social Interaction Anxiety Scale (SIAS)) and minor positive, negative and depressive symptoms (Community Assessment of Psychic Experiences (CAPE));
5. Symptoms of cybersickness (Simulator Sickness Questionnaire (SSQ)) [41].

Figure 1.2. Virtual reality study design
Participants were exposed to each of 5 conditions in the VR social environment for 4 minutes per condition in a random order. Measures were taken before (T0), during (T1) and after (T2) exposure to the VR environment.
Next, participants were exposed to the VR social environment (T1). By manipulating the variables (number, ethnic appearance and hostility of avatars), we could create five different experimental conditions in the social environment (A–E) with 0-3 social stressors (Table 1.1). In the experiment, participants were exposed to all five conditions in a random order, although the last condition always had at least two stressors. Each condition lasted 4 minutes. Before each condition, participants were asked to rate their current subjective distress on a scale from 0 to 100. After each condition, participants were asked to rate their maximum subjective distress during the condition and their current subjective distress. Their paranoid thoughts about avatars were also assessed using the State Social Paranoia Scale (SSPS) after each condition. After completion of the last virtual reality condition, another sample of blood was drawn. Symptoms of cybersickness were assessed and participants’ “presence” in the virtual reality world was measured using self-report questionnaires.

### Table 1.1. Virtual reality conditions.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Number of avatars</th>
<th>Ethnicity</th>
<th>Hostility</th>
<th>Number of stressors</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>6</td>
<td>80% own'</td>
<td>Neutral</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>40</td>
<td>80% own'</td>
<td>Neutral</td>
<td>1</td>
</tr>
<tr>
<td>C</td>
<td>40</td>
<td>80% 'other'</td>
<td>Neutral</td>
<td>2</td>
</tr>
<tr>
<td>D</td>
<td>40</td>
<td>80% own'</td>
<td>Hostile</td>
<td>2</td>
</tr>
<tr>
<td>E</td>
<td>40</td>
<td>80% 'other'</td>
<td>Hostile</td>
<td>3</td>
</tr>
</tbody>
</table>

*Number, ethnic appearance, and hostility of avatars were manipulated to create conditions A, B, C, D and E.

### Appendix 2. Immunology for dummies

The immune system has evolved to protect the host from pathogens, but it also has important roles in maintaining homeostasis. A key ability of the immune system is to distinguish self from non-self. The immune system comprises two subsystems, the innate immune system and the adaptive immune system. A simplified overview of the immune system is provided for readers who are less familiar with immunology. It focusses on the cell types and cytokines that are described in this thesis. It is important to know that the effect of any mediator is largely dependent on the context.

**Immune players**

Immune system includes physical barriers (e.g. skin, mucosal tissues), lymphoid organs, cells, humoral factors, and cytokines [42]. Different leukocyte subsets are distinguished by the glycoprotein differentiation antigens on their membranes; these are given cluster of differentiation (CD) numbers. Cytokines are small molecular weight messengers secreted by a cell to alter its own behaviour or that of another cell, i.e. cytokines modify or regulate cell functions. They are produced by virtually all types of cells and have a wide range of functions. Cytokines that are produced by leukocytes and have effects mainly on other leukocytes are called interleukins (e.g. IL-6, IL-12). Cytokines with chemoattractant functions are called chemokines (named mainly CXC or CC), while cytokines that interfere with viral replication are called interferons (e.g. IFN-γ).

**Innate immunity**

The innate immune system provides an immediate, but non-specific response to pathogens. It uses pathogen-recognition receptors to determine pathogen-associated molecular patterns and damage-associated molecular patterns. Cells produced by the innate immune response include macrophages, neutrophils and dendritic cells. These cells are able to engulf or digest pathogens or particles by phagocytosis. Macrophages and dendritic cells can present pathogen-derived peptides in Major Histocompatibility Complex (MHC) molecules to T cells. Furthermore, they express co-stimulatory molecules and secrete a wide array of cytokines. This is important in propagating, shaping and regulating the innate and adaptive immune responses. Macrophages can adopt different phenotypes, depending on the nature of the signals received when they are first activated. Classically activated macrophages produce large amounts of IFN-γ, IL-6, IL-12 and TNF-α as a pro-inflammatory response [43]. Alternatively, macrophages can be induced by IL-4, IL-10 and IL-13, and express IL-10, TGF-β and IL-1 receptor antagonists as a regulatory or anti-inflammatory response [44].
Natural killer (NK) cells are a type of white blood cell and have a critical role in the innate immune system, but they also have characteristics similar to the adaptive immune system. They are able to destroy compromised host cells, such as tumour cells or virus-infected cells by recognition of a condition known as “missing self”.

Adaptive immunity
Adaptive immunity is provided by T and B cells with antigen-specific receptors. The adaptive immune response system produces a specific response to a pathogen, but may take several days to develop. B and T cell receptors are generated randomly, resulting in millions of different naive B and T cells. Activation, multiplication and differentiation occur when a B or T cell encounters an antigen “cognate” (fitting) to its unique signal and receives a complementary signal. The complementary signal can be provided by co-stimulatory receptor/ligand interactions and by cytokines. B and T cells can differentiate into long-lived “memory cells” that can be rapidly activated upon re-infection. Thus, response to subsequent exposures is much faster, although still not as immediate as the innate immune system’s.

T cells are divided into several subtypes with different phenotypes and functions. Firstly, effector (CD8+) and helper (CD4+) T cells are distinguished. Effector or cytotoxic T cells are directly toxic to cells bearing their antigen, providing control of intracellular infections. Effector CD8+ T cells can produce multiple cytokines, including IFN-γ and IL-2.

Helper (CD4+) T cells are important orchestraters of the immune response and can differentiate into subsets depending on the nature of the signals present at the site of activation. Traditionally, T helper (Th)1 and Th2 cells are recognized [45]. Differentiation of naive CD4+ T cells into Th1 cells is induced by IL-12 and differentiation into Th2 cells by IL-4. Th1 cells stimulate cell-mediated immunity, cytotoxic T cells and macrophages by secreting IL-2 and IFN-γ. Th2 cells stimulate humoral (antibody-mediated) immunity by activating and stimulating B cells. B cells then undergo cell division and are able to secrete antibodies that can neutralize challenges directly or mark pathogens for destruction by complement for phagocytes. The main Th2 cytokines are IL-4, IL-5, IL-9 and IL-13.

More T cell subsets were recognized later, including regulatory T cells (Treg) and T helper 17 cells [46]. The combination of TGF-β and IL-6 induce differentiation into Th17 cells [47], which produce IL-6 and IL-1 cytokines and help to recruit neutrophils in the adaptive response to extracellular bacteria. Th17 cells also direct the destructive inflammatory response that is part of many autoimmune diseases. Regulatory T cells are characterized by surface expression of CD25 and nuclear expression of FoxP3, and they secrete immunomodulatory IL-10 and TGF-β cytokines and dampen immune response [48].

Figure 1.3. The immune system.
Overview of the cells that are examined in this thesis and involved in the innate and adaptive immune systems.
References