Abnormal brain connectivity in schizophrenia and bipolar disorder – a resting state functional MRI study

Kristina Cecilie Skåtun

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Division of Mental Health and Addiction, Oslo University Hospital
Institute of Clinical Medicine, University of Oslo
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LIST OF PAPERS

Paper I:

Paper II:

Paper III:
ABSTRACT
Schizophrenia and bipolar disorder are severe mental disorders, characterized by hallucinations, delusions, mood disturbances, and cognitive difficulties. Although these illnesses are divided into separate diagnostic categories, they have partly overlapping clinical characteristics and genetic risk factors. Despite the severity of these disorders, the underlying pathological mechanisms remain largely unknown, though one promising target to enhance our understanding of the pathophysiology is characterizing alterations in brain structure and function. Identifying and describing pathophysiological mechanisms is important in order to advance diagnostics, clinical care and treatment strategies.

Physician and anatomist Wernicke suggested already in the late 19th century that schizophrenia may be associated with disrupted connectivity between regions in the brain (Wernicke, 1906). However, it is not until the last few decades with the development of in vivo neuroimaging that studies have been able to show abnormalities in brain structure and function in these patient groups. Of particular interest, connections between brain regions (i.e. at the brain network level) are particularly affected (Fornito et al., 2012; Palaniyappan & Cousins, 2010; van den Heuvel & Fornito, 2014), supporting the dysconnectivity hypothesis of psychotic disorders (Friston, 1998; Stephan et al., 2009). Despite numerous imaging studies there are still discrepancies in the findings, possibly due to different methodological approaches and small sample sizes, and the diagnostic specificity of schizophrenia and bipolar disorder remains unclear.

For this reason, this thesis’ overall aim was to increase our understanding of functional brain connectivity in patients using a large subject sample, characterize abnormal connections and unique and overlapping features between schizophrenia and bipolar disorder, and to assess the clinical utility of altered brain functional connectivity by individual classification of patients and controls. Specifically, the aims of this thesis were 1) to probe the importance of brain network nodes using eigenvector centrality in schizophrenia and bipolar disorder, 2) investigate the role of the thalamus in abnormal brain connectivity in both patient groups, 3) to identify altered interactions between brain networks in schizophrenia patients and to assess the reliability and classification accuracy using machine learning across independent samples.

To this end, resting state functional MRI, which involves scanning the brain while the subject is relaxing without falling asleep, was collected from a large sample of schizophrenia and bipolar disorder patients and healthy controls. Project participants were included at the
NORMENT Centre at the Oslo University Hospital, with additional samples from Karolinska Institute in the third paper. Functional brain connectivity was examined using data-driven and multivariate analyses, primarily using a centrality measure from graph theory and independent component analysis with subsequent temporal correlation analyses.

Results showed disrupted functional connectivity in patient groups, with stronger effects in the schizophrenia patients. In paper I, decreased centrality in subcortical structures (hippocampus/amygdala and putamen) in both patient groups was found, while schizophrenia patients additionally showed reductions in visual and somatosensory regions. Five clusters yielded a pattern of increased centrality in frontal and parietal regions encompassing parts of the default mode network, with intermediate effects in bipolar disorder. In paper II, results showed schizophrenia patients to have reduced within-thalamic connectivity and thalamo-frontoparietal coupling and increased connectivity with sensory regions, while bipolar disorder patients showed increased thalamo-somatomotor connectivity. In paper III, reduced connectivity was found between several regions in schizophrenia, including frontal, sensory, and subcortical networks, and this was consistent across three samples. Moreover, the classification accuracy in the independent samples was up to 80%.

In conclusion, the thesis has demonstrated altered functional brain connectivity in patients with schizophrenia and bipolar disorder, with more substantial changes in schizophrenia patients, with converging evidence of altered connectivity in sensory, frontal, limbic, and thalamic regions. All three papers implicated sensory and frontal regions in schizophrenia, suggesting both aberrant sensory processing and deficits in regions important for cognition. From the second paper, it seems likely that the thalamus plays an important role in this observed dysconnectivity in patients, where a disrupted thalamic communication flow to the frontal lobe may affect higher-order cognitive processing, and where increased connectivity to sensory regions may be responsible for some of the sensory disruptions. Also, reduced limbic connectivity in both patient groups highlights the deficiencies in the brain’s mood-circuits. Lastly, the connectivity changes in paper III were robust across two scan sites with good classification accuracies, indicative of common brain alterations in schizophrenia and supporting that the clinical sensitivity of the brain network connectivity measures is generalizable across samples and scanners. Overall, this thesis demonstrates that schizophrenia and bipolar disorder are characterized by deficient information processing between sensory, limbic, thalamic, and frontal regions. These findings provide increased knowledge about pathophysiological mechanisms.
**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ANCOVA</td>
<td>Analysis of covariance</td>
</tr>
<tr>
<td>AP</td>
<td>Antipsychotic medication</td>
</tr>
<tr>
<td>BOLD</td>
<td>Blood Oxygen Level Dependent</td>
</tr>
<tr>
<td>DDD</td>
<td>defined daily dose</td>
</tr>
<tr>
<td>DMN</td>
<td>default mode network</td>
</tr>
<tr>
<td>DSM</td>
<td>The Diagnostic and Statistical Manual of Mental Disorders</td>
</tr>
<tr>
<td>ECM</td>
<td>Eigenvector centrality mapping</td>
</tr>
<tr>
<td>EPI</td>
<td>echo planar imaging</td>
</tr>
<tr>
<td>FIX</td>
<td>FMRIB’s ICA-based Xnoiseifier</td>
</tr>
<tr>
<td>fMRI</td>
<td>functional magnetic resonance imaging</td>
</tr>
<tr>
<td>GLM</td>
<td>general linear model</td>
</tr>
<tr>
<td>Hb</td>
<td>Haemoglobin</td>
</tr>
<tr>
<td>ICA</td>
<td>independent component analysis</td>
</tr>
<tr>
<td>NOS</td>
<td>not otherwise specified</td>
</tr>
<tr>
<td>PANSS</td>
<td>positive and negative syndrome scale</td>
</tr>
<tr>
<td>SA</td>
<td>Schizoaffective</td>
</tr>
<tr>
<td>SCID</td>
<td>structured clinical interview for DSM-V</td>
</tr>
<tr>
<td>SFF</td>
<td>Schizophreniform</td>
</tr>
<tr>
<td>TE</td>
<td>echo time</td>
</tr>
<tr>
<td>TFCE</td>
<td>threshold-free cluster enhancement</td>
</tr>
<tr>
<td>TR</td>
<td>repetition time</td>
</tr>
<tr>
<td>tSNR</td>
<td>temporal signal to noise ratio</td>
</tr>
<tr>
<td>VBM</td>
<td>voxel based morphometry</td>
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1. INTRODUCTION

1.1 Schizophrenia and bipolar disorder

Schizophrenia and bipolar disorder are severe mental disorders with devastating effects on the individual, their families, and society. These disorders are leading causes of morbidity, with poorer functioning and increased risk of suicide, and some of the most costly human illnesses (Miller et al., 2006; Whiteford et al., 2013). The combined life time prevalence of schizophrenia and bipolar disorder is estimated to be 2-3% of the population worldwide, though estimates vary across studies (McGrath et al., 2008; Merikangas et al., 2011).

The German psychiatrist Emil Kraepelin first described schizophrenia as *Dementia Praecox* in 1899 (Kraepelin, 1917). The illness was characterized as having an early onset with cognitive deterioration and an unfavorable outcome. Swiss psychiatrist Bleuler later introduced the term schizophrenia in 1911 (Bleuler, 1950). For bipolar disorder, states of depression and exaltations were described already by Hippocrates (460-337 BC) in ancient Greece. In 19th century France, Falret and Baillarger hypothesized that mania and depression were part of the same disease (Haustgen & Akiskal, 2006), and some 50 years later Kraepelin unified all types of affective disorders into manic-depressive insanity, which is considered separate from dementia praecox (Kraepelin, 1917). The Wernicke-Kleist-Leonhard school later distinguished unipolar disorder from bipolar disorder in the mid-20th century (Angst & Marneros, 2001), and the term bipolar disorder displaced manic-depressive illness in 1980 in the Diagnostic and Statistical Manual of Mental Disorders (DSM) III. Although the two disorders share common features, they are still categorized as separate diseases.

The first symptoms of schizophrenia and bipolar disorder usually occur in adolescence or early adulthood (Grande et al., 2015; Owen et al., 2016). For schizophrenia, symptoms include psychosis (loss of contact with reality), negative symptoms (such as social withdrawal and flattening effect), and cognitive dysfunction, while bipolar disorder is mainly characterized by episodes of elevated mood and depression. However, there is also overlapping symptomatology, where bipolar type I patients may experience psychosis in the manic episodes, and mood disturbances may be present in schizophrenia patients. Both are highly heritable brain disorders (Owen et al., 2016), with evidence pointing to altered connections between regions in the brain as disease mechanisms (Fornito et al., 2012; van den Heuvel & Fornito, 2014).

Several fields of study have worked towards understanding the underlying mechanisms of schizophrenia and bipolar disorder, inducing animal models, genetics, clinical
characterization, and brain imaging. These studies will be of great importance in improving diagnostics and subsequent treatments. However, despite extensive research and the identification of several risk factors and disease associated functions, the underlying pathological mechanisms of schizophrenia and bipolar disorder remain largely unknown. Thus, the diseases are still diagnosed based on descriptive criteria (symptoms and behavior).

Methodological advances in neuroimaging in the past decades allow for in vivo characterization of brain structure and function, which could potentially identify important biomarkers of these two disorders. Thus, in the current thesis, advanced imaging methods were used to study the functional network organization in the brain in both schizophrenia and bipolar disorder. In the subsequent sections, the clinical characteristics of these disorders will be described, followed by an outline of magnetic resonance imaging (MRI), brain networks and connectivity analytical approaches, and the status of the current knowledge in the field of brain network alterations in these patient groups.

1.1.1 Diagnosis and clinical characteristics
Schizophrenia and bipolar disorder are characterized by a range of symptoms, some of which are overlapping, including psychosis, mood alterations, and cognitive difficulties. The DSM-V (American Psychiatric Association, 2013) is a standard classification of mental disorders where the criteria for each diagnosis is described.

Schizophrenia
For schizophrenia, symptoms are diverse, and can be classified into positive, negative, disorganization, cognitive, mood, and motor symptoms (Tandon et al., 2009). Positive symptoms reflect added functions that are not expected to occur naturally. These include hallucinations, like hearing voices, and delusions, which are unrealistic thoughts that range from unfounded paranoia to ideas that your thoughts are broadcasted to people around you. Negative symptoms are manifested as a decrease or loss of normal functions, such as flattening effect reflected as diminished or no expression of emotions, lack of motivation, and social withdrawal. Disorganized speech and behavior include incoherent and illogical thoughts and speech, as well as odd behavior including awkward gait or unusual postures. Additionally, schizophrenia patients have often impaired neurocognitive functioning, including difficulties with abstract thinking, memory, attention, and planning. Mood symptoms, such a depression, are often associated with schizophrenia, and can typically
occur in the prodromal phase of the illness or after a psychotic episode. Motor activity is also affected, manifested as a slowing of psychomotor activity (associated with negative symptoms), or excessive purposeless movement (associated with positive symptoms).

The diagnostic criteria in DSM-V for schizophrenia are at least 1 month of active symptoms, including minimum two of the following: delusions, hallucinations, disorganized speech, grossly disorganized or catatonic behavior, and negative symptoms. These symptoms must also be so severe that they cause occupational or social dysfunction, and continuous signs of the disorder must be present for at least 6 months.

Other psychotic disorders have similar symptomatology, but do not fulfil all the criteria for schizophrenia. Schizoprophreniform (SFF) disorder exhibits the same symptoms as schizophrenia, but they are not necessarily impairing functioning, and duration is less than six months. Schizoaffective disorder (SA) has a mood episode co-occurring with the active symptom phase, but also minimum 2 weeks of symptoms not coinciding with the mood episode. Psychotic disorder not otherwise specified (NOS) does not fulfil criteria for any of the psychotic disorders, or there is lacking or contradictory information to set a specific diagnosis.

**Bipolar disorder**

Bipolar disorder is characterized by periods of elevated mood or depression. Characteristics include at least one hypomanic or manic episode, with symptoms of abnormally and persistently elevated, irritable or expansive mood. Manic episodes are persistent for minimum one week, cause impairments in social and occupational functioning, and have additional symptoms such as grandiosity, increased goal-directed behavior, racing thoughts, and a decreased need for sleep. Hypomania is a milder form of manic episodes not accompanied by delusions or hallucinations, and that does not cause marked impairments in functioning. These episodes of elevated mood are preceded or followed by a major depressive episode, lasting at least two weeks. Depressive symptoms encompass altered appetite or sleep, decreased energy, feeling worthless, and thoughts of death. Criteria for bipolar disorder type I is at least one manic or mixed episode, while bipolar disorder type II must have at least one depressive episode and one hypomanic episode. Bipolar disorder NOS diagnosis can also be given if symptoms do not fit clearly into the other subtypes.
1.1.2 Etiology and pathobiology

Despite extensive research, it is still not known what causes schizophrenia or bipolar disorder, though it is likely to be a combination of genetic, environmental, neurochemical, and neurobiological factors.

Genetic risk factors

Schizophrenia and bipolar disorder are both brain disorders with high heritability, where genetic variation accounts for 60-80% of the liability (Lichtenstein et al., 2009; Owen et al., 2016). Several genetic studies have identified genes associated with an increased risk for psychosis (Chen et al., 2015; Moskvina et al., 2009; Ripke et al., 2014; Ripke et al., 2013; Sklar et al., 2011). These disorders are polygenic, meaning that many genes contribute to these disorders’ development, but each gene only explains a small portion of the variance, making them hard to identify and target (Chen et al., 2015). However, advances in methodologies and pooling of data allow for the identification of disease associated genetic variants without a-priori hypotheses. These genome-wide association studies (GWAS) have identified several genetic variants in patients, thus identifying targets for further study (Chen et al., 2015; Maletic & Raison, 2014). Emerging evidence shows an overlap of both genetic architecture (Andreassen et al., 2013) and clinical phenotypes in schizophrenia and bipolar disorder, indicating that these two disorders are part of an etiological continuum with overlapping biological substrates (Brandt et al., 2014; Simonsen et al., 2011; van Os & Kapur, 2009; Williams et al., 2011).

Environmental risk factors

Several environmental factors have also been identified as risk factors for these disorders. For schizophrenia, these include childhood trauma, migrating to a new country, use of cannabis, growing up in an urban environment, and pre- and perinatal complications (Owen et al., 2016). There are similar risk factors for bipolar disorder, including cannabis and drug use, maternal influenza during pregnancy, and emotional stress and abuse during childhood (Marangoni et al., 2016). As there is an interaction between genes and the environment, they should not be looked at separately; an individual with genetic vulnerability may be more sensitive to environmental risk factors for psychosis, highlighting a gene-environment interaction (van Os et al., 2008).
Neurotransmitters

The neurochemical balance in the brain has been shown to be affected in schizophrenia and bipolar disorder. In schizophrenia, studies have shown increased pre-synaptic synthesis of L-dopa, the precursor molecule to dopamine, as well as an increased dopaminergic release at the synaptic cleft (Howes & Kapur, 2009). A modest increase in D2 receptors have also been found in the striatal region in schizophrenia patients, while genetic studies have identified several disease associated gene variants directly involved in the dopaminergic pathway (Howes & Kapur, 2009; Ripke et al., 2014). Glutamate and GABA have also been shown to be affected in schizophrenia patients (Howes et al., 2015). In bipolar disorder, several modalities have indicated a dysregulation of glutamate, and some studies also implicate GABA (Maletic & Raison, 2014). Due to the effect of lithium on treatment and prevention of affective episodes, intracellular signal transduction has been suggested to be affected (Alda, 2015).

Neurodevelopment

Schizophrenia is believed to be a subtle disorder of neurodevelopment, where the illness manifested in adulthood is proposed to have its origins in disturbed development of the nervous system (Owen et al., 2011). Studies have indicated altered development already in the late first or early second trimester, and obstetric complications and prenatal viral or bacterial infections are increased in schizophrenia (Fatemi & Folsom, 2009). Furthermore, schizophrenia share genes with disorders characterized as neurodevelopmental, such as autism (Owen et al., 2011), and there are large structural and functional brain changes observed in this patient group, even at the onset of the illness (Fornito et al., 2012; Pettersson-Yeo et al., 2011). Cognitive and motor difficulties have also been observed in patients as children, before the development of schizophrenia. Indications of abnormal neurodevelopment have not been associated with bipolar disorders.

1.1.3 Treatment and prognosis

Schizophrenia

Treatment approaches for schizophrenia includes the use of medication to alleviate symptoms. Antipsychotic medication (AP) targets the dopamine system in the brain, in particular by blocking D2 receptors (Howes et al., 2015). When these receptors are blocked, dopamine can no longer bind to and activate the receptors, resulting in a diminished
excitation. APs can alleviate positive symptoms in schizophrenia, but they have little or no impact in treating negative symptoms, and some patients also remain treatment-resistant (Owen et al., 2016). Several cognitive and psychosocial interventions also exists, including cognitive behavior therapy and cognitive and social skills training (Tandon et al., 2010). The prognosis of schizophrenia patients is very variable, though 20-50% of the cases show a fairly good outcome (Owen et al., 2016). However, while some patients recover and are never readmitted, others have chronic psychotic symptoms and need continuing support to function.

**Bipolar disorder**

Bipolar disorder patients can be treated with lithium and mood stabilizers, and bipolar disorder I patients with psychosis are also often treated with AP. Bipolar disorder is a chronic and highly recurrent disorder, where studies have showed up to 50% relapse of patients within 2 years (Vazquez et al., 2015), and approximately 70% within 5 years (Gitlin et al., 1995).

### 1.1.4 The need for biomarkers

A biomarker is a measureable indicator of the presence of a disease and/or its severity. For several diseases, such as cancer, diagnosis is based on an objective test targeting a biomarker in the patient. If the biomarker is present, the individual is given the associated diagnosis and subsequent treatment. However, for mental disorders the clinical tests and diagnoses are still based on descriptive criteria including subjective reports and interviews, and not a biological substrate. For this reason, it is sometimes hard to distinguish between similar disorders, and the diagnosis may also change with time.

Despite the severity of schizophrenia and bipolar disorder and a general poor outcome, the pathophysiologies of the illnesses remain mostly unknown, and the current treatment is not based on pathological substrates, such as abnormalities in neurotransmission, gene expression, and brain structure and function, but is discovered with serendipity. However, several studies have shown abnormalities in brain structure and function in schizophrenia and bipolar disorder, where connections between brain regions are particularly affected (Fornito et al., 2012; Palaniyappan & Cousins, 2010; van den Heuvel & Fornito, 2014). Altered brain structure or function may be potential biomarkers useful in setting an objective diagnosis in patients. However, despite numerous imaging studies there are still discrepancies in the findings and many questions remain to be answered (Pettersson-Yeo et
al., 2011). For this reason, the present thesis aims to increase our understanding of functional brain connectivity in patients, characterize abnormal connections and unique and overlapping features between schizophrenia and bipolar disorder, and assess the clinical utility of altered brain functional connectivity to correctly classify patients from healthy individuals. Identifying the underlying disease mechanisms is of vital importance to advance diagnostics, clinical care and develop novel treatment strategies.

1.2 Magnetic resonance imaging

MRI techniques have revolutionized our understanding of human brain function, due to its ability to depict brain structure and function in vivo. Images of the brain, or any other part of the body, are obtained by using the body’s natural magnetic properties. Typical brain MRI sequences can be divided into three broad categories; sequences sensitive to grey matter, diffusion tensor imaging sequences for capturing white matter tracts, and functional MRI (fMRI) sequences sensitive to changes in blood flow.

1.2.1 Structural MRI

MRI uses the properties of hydrogen in the brain to obtain an image. Hydrogen atoms make up water molecules (H₂O) and are abundant in the tissues of the brain, making it an ideal target for MRI. The hydrogen protons spin around their own axis, and, just like the earth, have two magnetic poles. Normally, the orientations of the axes of the poles are randomly orientated, but when placed in a strong magnetic field the protons’ axes will align with the field. A 3 Tesla scanner will produce a magnetic field 60,000 times stronger than the Earth’s. In addition to the main magnetic field, gradients are used to determine the location of the hydrogen atoms in the brain. The MRI scanner emits pulses of energy in the form of radio waves from its radiofrequency coils. The hydrogen atoms will absorb the energy, raising them to an excited state. Once the radiofrequency pulses are switched off, the protons will emit electromagnetic waves as they return back to their normal state. There are two different relaxation measures can be obtained when the protons return to their normal state. The first is the T1 relaxation, which is the time for magnetic vectors to return to their normal state, and the second is T2 relaxation, which is the time needed for the axial spins to return to their resting state. These signals can be detected by receiver coils, which are then used to construct an image. Different tissues return to their normal states at different rates, making it possible to obtain a contrast between different tissue types.
1.2.2 Functional MRI

fMRI gives an indirect measure of neuronal activity by detecting changes in blood flow responding to neuronal activity. Neurons are dependent on a flow of oxygen and glucose in order to function. As neuronal activity increases, there is an increased demand for both glucose and oxygen. To this end, the regional cerebral blood flow to the active region is increased. Haemoglobin (Hb) is the oxygen transporter in the blood, with four “seats” available for oxygen molecules. Its magnetic properties changes according to its level of (de)oxygénation; when a fully oxygenated Hb (diamagnetic) becomes deoxygenated (paramagnetic), a magnetic momentum is created that can be detected (Huettel et al., 2004). This is referred to the Blood Oxygen Level Dependent (BOLD) signal (Ogawa et al., 1990).

When neurons start to fire in a particular part of the brain, they use oxygen to generate the energy for an action potential. Oxygen then detaches from Hb, and diffuses from the blood capillaries and into the adjacent nerve tissue. To compensate for the deoxygenation in the blood, the brain directs a greater blood flow to the activated region, but overcompensates so that a surcharge of oxygenated blood arrives. This is referred to the hemodynamic response, which peaks after 4-6 seconds and is much slower compared to the neuronal signal (measured on the scale of milliseconds).

In fMRI, the main interest is activation over time, also referred to as temporal resolution. The time taken to scan the brain once can range between 1-3 seconds (repetition time, or TR), and repetitive scans are taken a few hundred times to obtain several data points over time. The temporal resolution comes at a cost of spatial resolution (how well one can distinguish changes over spatial locations in an image). Just as a picture is a compilation of pixels, brain images are built up of voxels that have three dimensions (usually between 1-4mm³ in size).

Although advances are now made using scanners with a higher magnetic strength (e.g. 7T), the resolution of MRI is on a much larger spatial scale than that of neurons. Voxels the size of millimeters contain several types of brain tissue and large populations of neurons, stressing the need to be careful in the interpretation of activation measured by fMRI. Moreover, it is important to remember that fMRI is a delayed and indirect measure of neuronal activity. Still, fMRI-studies have provided invaluable information about the brain’s functions, and as the methodology continues to improve we are likely to gain further insights about the brain in the near future using this approach.
1.2.3 Resting state fMRI

Resting state fMRI is an important method for investigating brain connectivity. Traditionally, fMRI studies have been used to investigate brain activity related to specific cognitive processes, such as working memory, when the subject has been exposed to a task or stimuli in the scanner. Resting state fMRI involves scanning of the brain while the subject is relaxing without falling asleep, and reveals large-amplitude fluctuations in the fMRI signal (Biswal et al., 2010; van den Heuvel & Hulshoff Pol, 2010). Participants are simply instructed to relax without falling asleep, while their brain activation is measured over time (usually 5-10 minutes).

Previously, the baseline brain activation was primarily of interest as a contrast for task activation, and was believed to be of little or no importance (Lowe, 2010). However, about 20 years ago, Bharat Biswal discovered that different brain regions exhibited spatially distributed networks at rest (Biswal et al., 1995). A region in the motor cortex was chosen as a seed in one hemisphere, and was shown to be correlated with the same region in the contralateral hemisphere. These correlations were largely in the resting state frequency. This was the first indication that brain activation at rest is organized and of great interest to study, and the number of resting state studies has since exploded. This method is advantageous due to its simplicity; it is not cognitively demanding, it’s quick and easy to implement, and no time is lost instructing the participants in the task. This is particularly useful with regards to patients, elderly, or children.

1.3 Brain networks and analysis approaches

Brain connectivity is an emerging field gaining a lot of interest, and aims at characterizing connections and interactions between regions in the brain. We can look at connectivity in terms of networks, which can be defined and described using graph theory. Here, a network is defined as consisting of nodes interconnected by edges (Bullmore & Sporns, 2009). Graph theory can be applied to any type of network. For instance, train stations (nodes) interconnected by railway tracks (edges), or a social network people (nodes) interconnected by friendship status on Facebook (edges). Thus, an edge does not necessarily have to be a physical link, but can be any type of defined interaction or relationship.

The exact same principles can be applied to the brain; the nodes can be defined as brain regions or structures, and the connections between them the edges. In structural networks, the edges can be the physical white matter tracts from one region to the next, much
like railway tracks connecting two stations. Since there are no physical links in functional networks, we need different measure to define the edges. In many functional brain network studies the edges are based on a statistical dependency between two regions, meaning if two regions’ activation patterns are correlated over time they are interconnected and share an edge (Bullmore & Sporns, 2009). The nodes can be defined in several ways, for instance at the voxel-level, as regions defined using a structural atlas, or as components from independent component analysis (ICA).

There are several ways to analyze resting state data. All have a basis in a brain region’s activation pattern over time, referred to that region’s time series. It is this information that forms the basis for further brain network analyses, usually in the form of statistically dependence between two regions’ time series, such as Pearson’s correlation. Connectivity can be studied by identifying connections between nodes within a single functional network, as well as the interaction between entire functional networks (functional network connectivity). Broadly, methods for analyzing RS-data can be divided into seed-based studies, ICA, and graph theory.

1.3.1 Seed analysis
Seed-analysis is a common method for investigating functional connectivity in the brain (van den Heuvel & Hulshoff Pol, 2010). It is hypothesis driven, where researchers may be interested in a particular structure or region in the brain and its connectivity. A region of interest is defined, such as the thalamus, and the mean time series for that region is obtained. The correlation between the region and other parts of the brain can then be calculated, resulting in a spatial map of brain regions with increased and decreased connectivity with the region of interest (Cordes et al., 2000; Jiang et al., 2004).

1.3.2 Independent component analysis
Independent component analysis (ICA) is a common approach for identifying and studying resting state brain networks. It is a data-driven approach, where brain activation patterns in the fMRI data are separated into multiple components, or networks, without any a-priori hypothesis (Calhoun & Adali, 2012). The spatial maps of the components are separated from each other and reflect regions with high degree of shared variance, meaning similar fluctuations in activation over time (Beckmann et al., 2005). This principle is similar to the “cocktail party” scenario, where the sound of several people speaking simultaneously is
separated into individual voices. In this way, ICA may discover hidden factors underlying multivariate data mixtures.

ICA-components largely reflect temporal correlations between regions that often work together (e.g. the primary visual network or higher order cognitive networks) (Friston et al., 1993; van den Heuvel & Hulshoff Pol, 2010). The networks have been shown to be robust, reliable, replicable, and there is an extensive overlap between brain networks observed during tasks and rest (Biswal et al., 2010; Greicius, 2008).

1.3.3 Graph theory
As described, nodes and edges facilitate the definition of a network (Bullmore & Sporns, 2009), but in order to describe and compare different networks to each other we need several properties that describe certain aspects of a network. Centrality is the simplest of these properties, measured by the number of edges connected to a node (referred to as node degree) (van den Heuvel & Sporns, 2013). Centrality measures probe the relative importance of nodes, where high-degree nodes (hubs) reflect essential processing units of a network. In the brain, centrality measures can identify brain regions exhibiting hub-like properties, reflecting critical modules in the processing systems of the brain (Joyce et al., 2010; Zuo et al., 2012).

Eigenvector centrality is another measure, which takes into account the degree of a node but also the degrees of its connecting nodes (Lohmann et al., 2010). This means that a node with a low degree can still have a high eigenvector centrality if its connecting nodes have many connecting edges. For a social network, this can be inferred as it’s not only how many you know, but also who you know that matters.

Path length is the smallest number of edges you have to pass through to get from node A to node B (Bullmore & Sporns, 2009). Path length indicates a network’s level of global integration; information can travel faster through a network with a shorter average path length. To this end, local efficiency of a node is the inverse of its average shortest path to connecting nodes. Clustering coefficient gives a measure of how probable it is that a node’s connecting nodes are also connected to each other. A high clustering is indicative of high interconnectedness, or local connectivity, of neighbouring nodes.

A network can be described using all the above mentioned properties and more. Connections may range from ordered to completely random, or somewhere in-between. Interestingly, several natural occurring networks are so-called “small-world”, combining high clustering with short path lengths (Bassett & Bullmore, 2006). This is believed to be an
optimal network configuration, both robust to loss of nodes whilst still efficient in information transfer.

1.4 Brain networks in schizophrenia and bipolar disorder

All the above mentioned analysis approaches provide a different way of defining and looking at brain networks, and how they differ in patient populations. Already in the late 19th century, psychiatrist and anatomist Wernicke suggested that schizophrenia may be associated with disrupted connectivity between regions in the brain (Wernicke, 1906). Swiss psychiatrist Eugen Bleuler first coined the term schizophrenia a few years later, meaning split (schizo) and mind (phrenia) (Bleuler, 1950). Even though brain connectivity was described over a century ago, it is not until the last few decades with the development of in vivo neuroimaging studies that this hypothesis has been further developed. Of particular interest, there is a growing notion that deficits in schizophrenia and bipolar disorder are due to pathological connections between brain regions (i.e. at the brain network level), and not just focal pathology in specific structures (Stephan et al., 2006). Today, the dysconnectivity hypothesis of psychotic disorders (Friston, 1998; Stephan et al., 2009) is widely known, and supported by numerous imaging studies (Fornito et al., 2012; Palaniyappan & Cousins, 2010; van den Heuvel & Fornito, 2014).

1.4.1 Structural alterations

Structural neuroimaging studies have found several alterations in schizophrenia and bipolar disorder. In schizophrenia, anatomical studies have shown a cortical thinning, reduced surface area of the cortex, enlargement of the ventricles, and reduced volumes in several structures (Rimol et al., 2010; Rimol et al., 2012). Bipolar disorder I patients also have some thinning of the cortex compared to healthy controls (Rimol et al., 2010). Subcortical structures have been shown to have a smaller volume in both schizophrenia and bipolar disorder, particularly in the hippocampus, thalamus, and nucleus accumbens (Hibar et al., 2016; Rimol et al., 2010). There is also evidence for reduced white matter volumes and integrity, where white matter tracts linking frontal, temporal, and parietal regions are affected in schizophrenia. In bipolar disorder, the fronto-parieto-temporal circuits are also affected, in addition to fronto-limbic connections (Brambilla et al., 2009; Heng et al., 2010).

Structural network organization is affected in schizophrenia, where diffusion imaging data have indicated increased clustering and modularity, which is indicated of more
segregated network organization (van den Heuvel & Fornito, 2014). Reduced centrality have been shown in nodes in medial frontal and parietal regions, and overlapping regions to the default mode network, indicating a reduced role of these regions in the brain network (Zhang et al., 2012). In fact, reduced centrality of hubs seems to be a hallmark of a range of brain disorders (Crossley et al., 2014). Path length is also longer, making communication less efficient between different parts of the brain. Studies in bipolar disorder are few, though evidence from diffusion tensor imaging point to increased path length, and reduced clustering coefficient and global efficiency, with particularly impaired integration between the hemispheres (Leow et al., 2013).

1.4.2 Functional alterations

Though functional connectivity is dependent on underling anatomical connections, there is not an easy one-on-one relationship, and two regions may have a strong functional correlation without an anatomical link. Functional connectivity can be measured both while performing cognitive tasks or during rest, and abnormal activation patterns in patients have been shown both in schizophrenia (Gur, 2011) and bipolar disorder (Cerullo et al., 2009; Chen et al., 2011). Abnormal functional connectivity have been found in regions or networks during rest, including the default mode network, the frontal cortex, the salience network, sensory regions, the thalamus, and limbic structures.

The default mode network

Several resting state networks have been identified and described in the last decade, most of them by using ICA-approaches or regions of interest of known coordinates. Most of these networks are depicting an increase in activation during a specific task. However, the only network that shows an increase in activation during wakeful rest is known as the default mode network (DMN). This network consists of the posterior cingulate cortex/precuneus, medial frontal and inferior parietal and temporal regions. The DMN is of great interest in psychiatric disorders because it has been linked to human cognition, including the integration of emotional and cognitive processing, internal mental processing, and monitoring of our surroundings (Deco et al., 2011; van den Heuvel & Hulshoff Pol, 2010).

The DMN is one of the most studied networks in schizophrenia. Most studies show altered connectivity within the DMN, though results vary and include findings of both hypo- and hyper-connectivity (Whitfield-Gabrieli & Ford, 2012). This may be due to a great variety
of methodological approaches, patient populations, and small sample sizes. The most common finding at rest, however, point to a hyperactivation of this network. During tasks, schizophrenia patients have shown a hyperactivation (meaning reduced task suppression) of the DMN and reduced anti-correlations with other networks or regions (Pettersson-Yeo et al., 2011; Whitfield-Gabrieli & Ford, 2012), suggesting a poorer differentiation between brain networks in patients. There are also some indications of altered DMN in bipolar disorder (Vargas et al., 2013), including reduced connectivity in the medial prefrontal cortex and abnormal recruitment of the basal ganglia (Ongur et al., 2010).

The prefrontal cortex
Several studies implicate systems involving the prefrontal cortex in schizophrenia, where most studies show reductions in functional connectivity (Fornito et al., 2012; Pettersson-Yeo et al., 2011). This region is essential for cognitive functions and executive control, including planning, working memory, decision making, and abstract thinking. The fronto-parietal network, also known as executive control and task-positive network, consists of lateral prefrontal and posterior parietal cortex and is involved in higher cognitive processes, including attention and working memory (Bressler & Menon, 2010; Cole et al., 2010). This network has been shown to be affected in schizophrenia (Cole et al., 2014), and is likely linked to the cognitive dysfunction observed in this patient group. In bipolar disorder, the inferior frontal cortex has been shown to be underactivated under emotional and cognitive processing (Chen et al., 2011).

The salience network
The salience network, also referred to as the cingulo-opercular network, consists of the anterior fronto-insular cortex and anterior cingulate cortex. It is involved in the detection of behaviorally relevant stimuli and switching between states of internal focus (DMN) and external stimuli (fronto-parietal network) (Sridharan et al., 2008; Uddin, 2015). Connectivity of the salience network is altered in schizophrenia (Palaniyappan et al., 2012), and could play a role in patients’ difficulty to separate external information from self-generated thoughts, manifested as delusions and hallucinations (Uddin, 2015). Evidence from bipolar disorder has shown reduced connectivity within this network, and altered coupling with the cerebellum (Mamah et al., 2013).
**Sensory networks**

There are several sensory networks in the brain, including those involved in processing information in visual, somatomotor, and auditory cortical regions. These have received less attention, even though sensory dysfunction has been well documented in schizophrenia in non-imaging studies (Butler et al., 2008; Javitt, 2009; Javitt & Sweet, 2015). Sensory modalities include auditory, visual, and somatomotor processes, with evidence pointing to affected sensory processing in all these modalities in schizophrenia (Butler et al., 2008; Javitt, 2009). The neuroimaging studies looking at sensory networks have also found a general reduced connectivity in these regions (Berman et al., 2016; Kaufmann et al., 2015; Liemburg et al., 2012).

**The thalamus**

The thalamus is a densely connected subcortical structure and is a relay station for motor and sensory information towards higher-order cortical processing (Herrero et al., 2002), and a facilitator of cortico-cortical communication (Sherman, 2007). In schizophrenia, neuroimaging have implicated alterations in thalamic activation, connectivity, shape and volume (Byne et al., 2009; Cronenwett & Csernansky, 2010; Smith et al., 2011a; van Erp et al., 2015). Several fMRI studies have found reduced thalamic-prefrontal connectivity and increased coupling with somatomotor regions in schizophrenia (Anticevic et al., 2014a; Cheng et al., 2015; Kaufmann et al., 2015; Woodward et al., 2012), both in early stage and chronic psychosis spectrum patients (Woodward & Heckers, 2015). In bipolar disorder, altered thalamic activation has been observed during cognitive and emotional processing (Cerullo et al., 2009; Delvecchio et al., 2012), in addition to altered connectivity between thalamus and striatum and parahippocampus at rest (Teng et al., 2014).

The thalamus is a heterogeneous structure with several nuclei, each with dense and specific cortical or subcortical connections (Herrero et al., 2002). Disruptions of nuclei circuits have been observed in schizophrenia, in particular the mediodorsal nuclei, anterior nuclei, and the pulvinar, each associated with a distinct thalamo-cortical connectivity profile (Byne et al., 2009; Lewis et al., 2001). Although studies have indicated graded alterations in bipolar disorder (Anticevic et al., 2014a; Anticevic et al., 2014b), there is also evidence of differential thalamic sub-regional cortical connectivity patterns in schizophrenia and bipolar disorder (Anticevic et al., 2014b).
Limbic structures

The limbic system consists of several structures and is involved in the processing of emotional information and long-term memory. Two of the limbic structures are the hippocampus (crucial for episodic memory) and the amygdala (emotional processing), both shown to be affected in schizophrenia (Heckers & Konradi, 2010; Rimol et al., 2010) and bipolar disorder (Frey et al., 2007; Garrett & Chang, 2008). Mood disturbances are characteristic features of both disorders, which could be due to disrupted circuits of the limbic system.

Network organization

Functional network organization is also altered in patients. Previous studies have demonstrated reduced connectivity of high-degree hubs in schizophrenia, especially in prefrontal and parietal regions, while other brain regions show an emergence of new hubs (Fornito et al., 2012; Lynall et al., 2010; van den Heuvel & Fornito, 2014). The rich-club organization (hubs connected to other hubs) is especially disrupted in schizophrenia, where decreased centrality of critical hubs may point to less centralized and coordinated brain network functioning (van den Heuvel & Fornito, 2014).

There is some discrepancy between structural and functional findings with regards to graph theory brain networks, where functional alterations are seemingly contradictory to those of structural alterations. Findings point to reduced clustering and modularity, and increased or unchanged global efficiency. There are several potential explanations for these discrepancies, differences in methodology, how the network nodes are defined, and the use of weighted versus thresholded graphs. Also, structural and functional alterations do not necessarily have to overlap, as altered functional networks could be a compensation for structural changes.

1.5 Aims

The main aim of the present thesis was to 1) identify abnormal functional brain connectivity in schizophrenia and bipolar disorder, and 2) to investigate these characteristics for similarities and differences between patient groups and in relation to healthy controls. To accomplish these goals, we conducted three studies with the following aims:
The *first aim* was to probe the importance of brain network nodes using eigenvector centrality to assess to what degree global brain connectivity at the voxel-level are overlapping between bipolar disorder and schizophrenia and different from healthy controls.

The *second aim* was to investigate thalamic brain connectivity in patient groups by characterizing within-thalamic, thalamo-cortical, and thalamic subregional functional connectivity across schizophrenia and bipolar disorder and to determine if thalamic structural variability could explain any functional alterations.

The *third aim* was to identify altered interactions between brain networks in schizophrenia patients, to assess the reliability of the effects across three independent samples, and to test to what degree these findings could be used to correctly classify patients and controls within and across samples.
2. MATERIALS AND METHODS

2.1 Setting and facilities

Images were acquired on a General Electric (Signa HDxt) 3T scanner (General Electric Company; Milwaukee, WI, USA) with an 8-channel head coil at the Section of Radiology and Nuclear Medicine, Oslo University Hospital. Participants were interviewed and all data was analyzed at NORMENT, Oslo University Hospital.

Paper III included two additional independent samples of schizophrenia and healthy controls from the Karolinska Institute, Stockholm, both of which were scanned at a 3T General Electric Discovery MR750 with identical sequences. Data was collected at the Karolinska Institute, and analyzed at NORMENT.

2.2 Study samples

2.2.1 The Norwegian sample

The current project is part of the Norwegian Centre for Mental Disorders Research (NORMENT), formerly known as the Thematically Organized Psychosis (TOP) Study Group. The centre runs a multidisciplinary large-scale program to study underlying mechanisms of psychotic disorders, focusing on clinical characteristics, neurocognitive functioning and brain biology as well as genetic factors. The organization recruits patients from all hospitals in Oslo to subprojects sharing a common protocol, biobank and database, and infrastructure for neurocognitive testing, MRI and molecular genetic lab service. Written informed consent was obtained from all participants and the study was approved by the Regional Committee for Medical Research Ethics South East Norway and the Norwegian Data Inspectorate.

The main patient groups included in the TOP study are patients with a schizophrenia and bipolar disorder spectrum disorder, recruited from psychiatric hospital units of both in- and out-patients in the Oslo area (Table 1). Patients completed a clinical interview with a trained clinician, including history of illness and symptom measures. Diagnostic criteria were assessed using the structured clinical interview for DSM-IV (SCID). Inclusion criteria for the patients were being able to fully understand all the information given to them, including that the study was voluntarily and they could withdraw at any time, and provide informed consent. This ability was judged by a clinical psychologist or by a physician trained in psychiatry.
Healthy controls aged 18-45 years were invited to participate based on a random selection from the Norwegian National Population Register. Healthy controls were interviewed with general questions about severe mental illness symptoms and the use of Primary Care Evaluation of Mental Disorders (PRIME-MD) (Spitzer et al., 1994), to ensure no history of a severe psychiatric disorder in controls or any of their close relatives. Controls with history of drug abuse/dependency or somatic conditions interfering with brain functioning were not included. Patients and controls with a history of head trauma, neurological disorders, or pathological neuroradiological findings were also excluded.

2.2.2 The Stockholm samples
Two samples were included from the Karolinska Institute, Stockholm, Sweden. The first, Karolinska Schizophrenia Project (KASP), consisted of recently diagnosed persons in the schizophrenia spectrum. The Human Brain Informatics (HUBIN) dataset consisted of older chronic patients from a 12-year follow-up. Patients were diagnosed using SCID by trained clinicians, and controls were evaluated using a structured clinical interview in HUBIN (Spitzer et al., 1986), and by the Mini-International Neuropsychiatric Interview in KASP (Sheehan et al., 1998). Both KASP and HUBIN samples were scanned at the same 3T GE scanner with the same sequence.

Table 1. Number of participants in each paper and overlap across papers.

<table>
<thead>
<tr>
<th></th>
<th>Paper I</th>
<th>Paper II</th>
<th>Paper III</th>
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<tr>
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</table>
2.3 Imaging acquisition and preprocessing

2.3.1 Imaging acquisition

Functional data

A T2*-weighted 2D gradient echo planar imaging (EPI) sequence with 203 volumes was collected [repetition time (TR)=2638ms; echo time (TE)=30ms; flip angle=90°; acquisition matrix=64x64; in-plane resolution=4x4mm; 45 axial slices; slice thickness=3mm]. The Stockholm sample used an EPI-sequence with 200 volumes [TR=2000ms; TE=27ms; flip angle=90°; acquisition matrix=128x128; in-plane resolution=1.875x1.875mm; 40 axial slices; slice thickness=3mm]. The three first volumes were discarded in addition to five dummy volumes to ensure homologous tissue magnetization. Subjects were instructed to lay still with their eyes open and the head was fixed with foam pads to reduce motion.

Structural data

Sagittal T1-weighted sequences were also collected for registration purposes for both the Norwegian sample [FSPGR sequence; TR=7800 ms; TE=2.956 ms; TI=450 ms, flip angle=12°; in-plane resolution=1x1mm; number of slices=166; slice thickness=1.2mm; acquisition time=7min 8s], and the Swedish sample [BRAVO sequence; TR=7904 ms; TE=3.06 ms; TI=450 ms, flip angle=12°; in-plane resolution=.94x.94mm; number of slices=146; slice thickness=1.2mm].

2.3.2 Preprocessing

T1-weighted data from all subject samples were processed using FreeSurfer (Dale et al., 1999). Automated full brain segmentation (Fischl et al., 2002) was used to provide precise brain extracted structural volumes used for co-registration of the fMRI volumes. All brain extracted datasets were visually inspected and manually edited if required. Subjects with too much motion for brain segmentation or other large scanner artifacts in their structural data were excluded.

Resting state fMRI data was processed using FEAT, part of FSL (Jenkinson et al., 2012), including brain extraction, motion correction, spatial smoothing using a Gaussian kernel of full-width at half-maximum (FWHM) of 6mm, and high pass filter of 100s. fMRI volumes were registered to the subjects’ structural scans using FMRIB’s Linear Image Registration Tool (FLIRT (Jenkinson et al., 2002)) implementing boundary-based registration (Greve & Fischl, 2009). The T1-weighted volumes were non-linearly warped to the Montreal...
Neurological Institute MNI152 template (Mazziotta et al., 2001) using FMRIB’s Nonlinear Image Registration Tool (FNIRT (Jenkinson et al., 2012)), and the same warping was applied to the fMRI data. All registered fMRI data were visually inspected. Subjects with scanner artifacts or large signal drop-out were excluded.

2.3.3 Data cleaning

In order to minimize confounding effects of motion and other sources of noise we performed single subject ICA and applied FMRIB’s ICA-based Xnoiseifier (FIX) with a standard training set (Griffanti et al., 2014; Salimi-Khorshidi et al., 2014). Using default options, components were classified as noise and non-noise variability, respectively, using a standard training set supplied with FIX (threshold: 20). Components identified as noise and the estimated subject motion parameters were regressed out of the data. ICA-based denoising has been shown to compare favorably to other data-cleaning methods (Pruim et al., 2015a; Pruim et al., 2015b).

In paper II and III we also assessed the effects of denoising on the data and its interactions with diagnosis by calculating the proportion of noise and variance removed, and the temporal signal to noise ratio (tSNR) (Roalf et al., 2016) before and after cleaning the data. A repeated measures ANOVA tested for main effects of denoising (pre and post FIX) and group (schizophrenia, bipolar disorder, and controls in paper II, and schizophrenia and healthy controls in paper III), and their interactions on tSNR.

2.4 Eigenvector centrality mapping (paper I)

In paper I, we defined the functional brain network based on graph theory using a centrality measure. The nodes were defined at the voxel-scale, and the whole brain was included in the network measure. Eigenvector centrality mapping (ECM) provides a weighted centrality index, taking into account degree centrality (the number of edges to a node), while favoring connections to high-centrality nodes (Lohmann et al., 2010). Defining voxels as nodes, ECM enables data-driven analysis of functional connectivity based on the voxel-wise correlation structure (Lohmann et al., 2010; Zuo et al., 2012).

Preprocessed, cleaned and normalized functional datasets were processed in Lipsia (Lohmann et al., 2001), and voxel-wise ECM volumes were calculated considering the absolute value of the correlation coefficient, i.e. assigning equal weights to positive and negative correlations. We used a common mask excluding voxels not represented in all
subjects to ensure that individual centrality maps were computed based on the same brain mask, and we calculated a one-sample t-test of ECM across healthy controls. Figure 1 illustrates two main analysis pathways used in the current thesis.

![Figure 1](image)

**Figure 1.** Two main analysis pathways for resting state data used in the current thesis. Time series data from all voxels in the brain are organized into individual spatial maps based on eigenvector centrality mapping (ECM) or independent component analysis (ICA). Spatial maps from ECM are tested voxel-wise for effects of group, as are voxels within an individual network from ICA. Between-network analysis correlates the time series from all components and tests for effects of group.

### 2.5 Independent component analysis and connectivity matrices (paper II and III)

#### 2.5.1 Independent component analysis (ICA)

Group ICA was used in paper II and III, where preprocessed and cleaned fMRI datasets were submitted to a temporal concatenation group spatial ICA using MELODIC in FSL (Jenkinson et al., 2012). In paper II, we used group ICA to decomposed the datasets into 40 spatially independent components (ICs), chosen on the basis that the putamen and thalamus separated into different components at this model order. Twenty-one non-noise components were used in further analysis. To avoid bias due to uneven sample sizes, randomly chosen preprocessed resting state fMRI volumes of 50 bipolar disorder patients, 50 schizophrenia patients and 50 controls were included in the decomposition.

Based on a recent study (Hale et al., 2015), we performed a second group ICA restricted to the left and right thalamus to decompose the data into sub-thalamic independent components, with a model order of 15 in each hemisphere (paper II). Five components were disregarded as noise in each hemisphere based on the group ICA spatial maps, and corresponding contralateral components were combined into one seed, yielding 10 sub-
thalamocortical components of interest. We performed separate decompositions for each hemisphere as this yielded better correspondence of contralateral clusters and overlap with known anatomical nuclei, shown to yield similar results as whole thalamus ICA (Hale et al., 2015). We employed dual regression on each unthresholded bilateral sub-thalamic seed separately, mimicking conventional whole-brain seed-based analysis.

In paper III, to facilitate the integration of fMRI data obtained from two different scanners in the same analysis, we employed a meta-ICA approach (Biswal et al., 2010). Briefly, we selected 140 subjects from each site and ran two decompositions per site (model order 80), each including 35 patients and 35 controls. A common brain mask containing voxels with signal in all subjects was used. The four resulting group-level ICA spatial maps were merged into a single 4D-file, and used as input for a single-session meta-ICA (Biswal et al., 2010) with a model order of 80. Components clearly associated only with scanner specific signals or artifacts were removed, as were components related to motion, white matter, and cerebrospinal fluid. Due to the restricted field of view, which limited the brain coverage, components primarily encompassing the cerebellum were excluded. In total, 59 components were submitted to further analysis.

Dual regression was used to estimate individual spatial maps corresponding to the group ICA (papers II and III) (Beckmann et al., 2009; Filippini et al., 2009). Briefly, the first step in dual regression uses the independent components as spatial regressors to estimate the components’ time courses for each individual. Next, the time courses are normalized by their variance and used as temporal regressors against the fMRI data set to find the individual spatial maps reflecting the functional connectivity maps specific for the relevant components for each participant.

2.5.2 Network connectivity matrices
In paper II and III, we evaluated the interaction between the independent components from the group ICA. In paper II, we only looked interactions between the thalamus and the other components, while in paper III we extended the analysis to include interactions between all components.

In line with several recent studies (Brandt et al., 2015; Kaufmann et al., 2015; Smith et al., 2015; Smith et al., 2013), we defined each of the components’ spatial maps as nodes in the brain network, and the estimated temporal associations between the nodes as their edges. After regressing out the time series of the components defined as noise, we computed
connectivity matrices for each individual dataset defined as the z-normalized node-by-node regularized partial correlations using a combination of custom-made Matlab (The Mathworks Inc) tools and functions made available in FSLnets (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLNets). In line with recent studies (Brier et al., 2015; Kaufmann et al., 2016), regularization was performed based on the Ledoit & Wolf theorem, allowing an estimation of the regularization strength (lambda) at the single-subject level (Ledoit & Wolf, 2003; Schäfer & Strimmer, 2005).

For paper II, we had 20 unique edges between the thalamus and 20 other independent components, while for paper III, correlations between all 59 components yielded individual connectivity matrices comprising 1711 unique edges.

2.6 Statistical analyses

After providing functional connectivity measures for each individual, we wanted to investigate any differences between diagnostic groups. Unless stated otherwise, for all analyses in paper I and II we performed F-tests to evaluate main effect of group (schizophrenia, bipolar disorder and healthy controls) on connectivity, with age and sex as covariates. For paper III, we tested for effect of group (schizophrenia and controls) with age, sex, and scanner (Oslo, Stockholm) as covariates.

For all voxel-wise analyses, statistical inference was done using permutation testing across 5000 iterations and threshold-free cluster enhancement (TFCE) to correct for multiple comparisons (Smith & Nichols, 2009). Other analyses were either corrected at the FDR- or Bonferroni-level.

2.6.1 Eigenvector centrality mapping (paper I)

Main effects of diagnosis on centrality were tested using general linear models (GLM). An F-test evaluated any differences between the three groups, and additional t-contrasts assessed pairwise group differences. Individual mean ECM values in clusters showing main effects of diagnosis were submitted to further analysis in SPSS in order to estimate commonly reported effect sizes. In order to evaluate whether clusters with increased/decreased ECM in patients were regions with high or low centrality, paired t-tests were run between means of each cluster and the ECM mean across the entire brain.

Effects on ECM reflect altered global voxel centrality, but do not reveal the origins (e.g., the degree to which the alterations are due to spatially distributed or specific
alterations). In line with previous studies, (Alnaes et al., 2015; Schoonheim et al., 2014) we used clusters with significant diagnostic effects on ECM as seeds in dual regression (Beckmann et al., 2009; Filippini et al., 2009) in order to investigate the regional contribution. These connectivity maps were concatenated across participants and submitted to permutation testing in order to explore the regional sources of the diagnostic effects on functional connectivity patterns as revealed by ECM.

2.6.2 Within-network functional connectivity (paper II)
In paper II, we investigated thalamic functional connectivity of the thalamic component from the group ICA, and on all the connectivity maps from the 10 thalamic subregions. Effects of diagnosis on all these thalamic spatial maps were tested voxel-wise in the whole brain using a GLM and permutation testing. We performed an F-test to assess main effects of group, and subsequent t-tests to probe the pairwise group differences.

2.6.3 Between-network functional connectivity (paper II and III)
For paper II, edge-wise effects of group on the 20 thalamo-cortical edges were tested using analyses of covariance (ANCOVAs), corrected for multiple comparisons using false discovery rate (FDR, q=.05), based on independence or positive dependence (Nichols & Hayasaka, 2003). We tested for differences between groups (schizophrenia vs controls, bipolar disorder vs controls, and schizophrenia vs bipolar disorder) in the identified edges using pairwise comparisons.

In paper III, in order to visualize the associations between the nodes, the full correlation matrix averaged across subjects was used to compute a hierarchical clustering of the components using the linkage and dendrogram functions in Matlab, where nodes are grouped according to the correlations between their respective time series. We tested for effects of diagnosis on edge connectivity for each edge in the correlation matrices, and corrected the results at both the FDR (Nichols & Hayasaka, 2003) and Bonferroni-level (p < .05/1711). For significant edges, we also tested if group effects were present within each of the three samples.

In paper III, whereas the main analysis is targeting the connections between the nodes, the relative involvement of each of the nodes is also of interest. In order to assess the cumulative importance of each of the network nodes in distinguishing between cases and controls, we calculated the eigenvector centrality of each node based on the edge-wise F-
values from the group ANCOVA. A high centrality indicates altered connectivity with several other nodes, indicating a relative importance of this specific node in the discrimination between cases and controls.

2.6.4 Structural analysis (paper II)

Left and right thalamic volume and estimated total intracranial volume (Buckner et al., 2004) were computed using FreeSurfer, and we tested for group differences in thalamic volumes using ANCOVAs covarying for age, sex, and estimated total intracranial volume. Regional gray matter in the thalamus was tested using voxel based morphometry (VBM) in FSL (Douaud et al., 2007). A gray matter template was created from 50 subjects in each diagnostic group using brain-extracted gray matter segmented images that were non-linearly registered to standard space. All structural images were then non-linearly registered to the template, and smoothed using an isotropic Gaussian kernel with a sigma of 2 mm. Group differences were tested voxel-wise only within the thalamus using the same GLM and permutation testing approach as for thalamic connectivity analyses.

We tested for group differences in thalamic shape using FIRST (Patenaude et al., 2011) in FSL. Thalamus was segmented, producing mesh and volumetric outputs. Vertex analysis was then performed on the reconstructed mesh in MNI space, aligning to mean thalamic shape across subjects, and removing effects of global size. This provides a measure of the location of each vertex in relation to average thalamic shape, where positive and negative values represent location outside and inside the average surface, respectively. Statistical inference was performed using permutation testing and TFCE optimized for 2D data.

In order to test if functional connectivity differences are explained by structural alterations, we extracted mean functional connectivity from a cluster with reduced thalamic connectivity in schizophrenia, and VBM values from a thalamic cluster with reduced gray matter. An ANCOVA tested for differences between schizophrenia and healthy controls on functional connectivity with age and sex as covariates, and in a second run VBM was included as an additional covariate.

2.6.5 Multivariate analysis – machine learning (paper III)

In paper III, we assessed the reliability of the connectivity fingerprint of schizophrenia across samples using regularized linear discriminant analysis classifiers (Friedman, 1989; Schäfer &
Strimmer, 2005) on edgewise connectivity strength, similar to the procedures in (Alnaes et al., 2015; Kaufmann et al., 2016; Kaufmann et al., 2015). First, we trained a binary classifier on the TOP sample to discriminate healthy controls from schizophrenia and tested the classifier on HUBIN, KASP as well as on the merged HUBIN+KASP sample. Second, since HUBIN and KASP were too small to form a robust training set for a classifier and since both data sets were acquired on the same scanner, we merged the samples and trained a classifier on HUBIN+KASP, which was then tested on the TOP sample.

2.7 Post hoc analyses

There are several potential confounders that might influence brain activation or morphology. These factors include demographic variables (like age and sex) and clinical variables (symptoms, duration of illness, medication, and different diagnostic subgroups). Motion is also an important potential confounder that could influence the findings, especially because patient populations tend to have more head motion than controls. To this end, we performed several post hoc analyses to evaluate whether these variables could explain the effect of group on connectivity, and if the clinical variables were associated with strength of effects in the patient groups. Similar tests were performed in the three studies, which are described in further detail below.

2.7.1 Associations with clinical variables

In paper I, we tested for associations with positive and negative syndrome scale (PANSS) for positive, negative, general psychopathology, and total scores (Kay et al., 1987) in each cluster within each patient group, with age and sex as covariates. Nominal p-values were adjusted using Bonferroni correction to control the family-wise error rate. Since symptom-connectivity associations do not necessarily comply with the clusters showing case-control differences, we also performed full-brain voxel-wise analyses testing for associations between symptoms and centrality within each patient group, with age and sex as covariates.

In paper II, mean connectivity in thalamic clusters with significant effect of group were submitted to ANCOVAs in SPSS, testing for associations with PANSS (Kay et al., 1987), and depression by the Calgary Depression Scale for Schizophrenia within each patient group (Bonferroni threshold; p < 0.0125). In paper III, associations with total PANSS score (TOP and KASP patients) and duration of illness (all three samples) were tested on the connectivity of edges showing main effects of diagnosis, covarying for age, sex and scan-site.
2.7.2 Medication

In all papers, dose of medication was calculated as the ratio of the defined daily dose (DDD) as described in the WHO guidelines (http://www.whocc.no/atcddd), allowing for the comparison across different antipsychotics.

In paper I, we tested for effects of medication by using an ANCOVA to compare ECM values between the patients with bipolar disorder who were treated with antipsychotic medication (n=22) and those who were not (n=15). Likewise, we tested for differences between schizophrenia and bipolar disorder groups after excluding patients who were not treated with antipsychotics (schizophrenia n=59, and bipolar disorder n=22).

In paper II, we extracted the mean functional connectivity of the thalamic cluster with significant difference between schizophrenia patients and controls, and tested for effect of antipsychotics on functional connectivity within the schizophrenia spectrum group. Within the bipolar disorder I group, effects of antipsychotics (22 patients) were tested on any significant edges. In paper III, we tested for effects of medication within the patient group on any significant edges identified in the main analysis, with age, sex and scan-site as covariates.

2.7.3 Subgroups

As we included a broad psychosis spectrum, we investigated if there were any differences between subgroups. In paper I, the ECM ANCOVAs were rerun after excluding patients with psychosis NOS, and then after excluding patients with SA and SFF disorders. We conducted an ANCOVA with age and sex as covariates to test for differences between schizophrenia and SA and SFF disorders and psychosis NOS. As the bipolar disorder group was too small to estimate reliable subgroup effects, we tested only for differences between bipolar disorder I and bipolar disorder II/bipolar disorder NOS.

In paper II, we extracted the mean functional connectivity of the thalamic cluster with significant difference between schizophrenia patients and healthy controls, and ran ANCOVAs testing for effect of group by first excluding psychosis NOS patients, and then also SA and SFF patients. For bipolar disorder, we tested for sub-group differences on any edge showing main effects of bipolar disorder by first only including bipolar disorder I patients, and then only bipolar disorder II and bipolar disorder NOS patients.
In paper III, we tested for effect of subgroup (98 schizophrenia, 27 SA, 5 SFF, psychosis 43 NOS, 4 brief psychotic disorder, and 6 delusional disorder) on the connectivity of edges showing main effects of diagnosis, covarying for age, sex and scan-site.

2.7.4 Effects of motion
For all papers, we calculated mean relative motion per individual (defined as the average root mean square of the frame-to-frame displacement). Although we went to great lengths to minimize the influence of participant motion using FIX and aggressively regressing the motion parameters out of the data, some residual effects could remain. Thus, we ran different analyses to test whether the effect of motion explained any group differences.

In paper I, we conducted an ANCOVA to test for differences in motion between groups, and each ECM model was rerun including average motion as an additional covariate. In paper II, effects of motion were tested in the significant thalamic cluster within the schizophrenia patients, and on any significant edges within the bipolar disorder I group. In paper III, motion was added as a covariate in the main analysis to evaluate if the effects of group remained after controlling for this variable.
3. RESULTS

3.1 Paper I

The human brain is organized into functionally distinct modules of which interactions constitute the human functional connectome. Accumulating evidence has implicated perturbations in the patterns of brain connectivity across a range of neurological and neuropsychiatric disorders, but little is known about diagnostic specificity. In our first study, we wanted to derive global functional connectivity using eigenvector centrality mapping, which allows for regional inference of centrality or importance in the brain network. Our primary aim was to identify common and unique global brain connectivity differences in schizophrenia and bipolar disorder, as well as patterns distinguishing the two patient groups from each other. We anticipated centrality reductions in hub regions across patient groups. Moreover, we hypothesized altered centrality in regions involved in sensorimotor and perceptual processing, the frontal cortex, cingulate, and parietal regions in schizophrenia, and aberrant limbic centrality in bipolar disorder.

Results showed regional differences in global resting state brain connectivity in schizophrenia and bipolar disorder patients compared to healthy controls in 12 clusters, with strongest effects seen in schizophrenia. Decreased centrality occurred in subcortical structures (hippocampus/amygdala and putamen) in both patient groups, while schizophrenia patients additionally showed reductions in visual and somatosensory regions, implicating reduced connectivity and poorer functioning in sensory and limbic structures in patients. Five clusters yielded a pattern of increased centrality in frontal and parietal regions encompassing parts of the default mode network, suggesting a system-level dedifferentiation of parts of this network in schizophrenia, with intermediate effects in bipolar disorder. Patient groups differed from each other in most cortical clusters, with strongest effects in sensory regions. Lastly, no significant associations with symptom domains were found.

3.2 Paper II

The thalamus is a highly connected subcortical structure that relays and integrates sensory and cortical information, which is critical for coherent and accurate perceptual awareness and cognition. Thalamic dysfunction is a classical finding in schizophrenia, and resting state functional MRI has implicated somatomotor and frontal lobe thalamic dysconnectivity. However, it remains unclear whether these findings generalize to different psychotic disorders, are confined to specific thalamic sub-regions, and how they relate to structural
thalamic alterations. To this end, the aim of our second study was to investigate how the thalamus plays a role in abnormal brain connectivity in patient groups by performing a comprehensive brain MRI investigation of thalamic resting state functional connectivity and structure in a large sample of schizophrenia and bipolar disorder patients. We hypothesized 1) reduced thalamic recruitment, 2) increased thalamo-somatomotor connectivity and decreased thalamo-frontal connectivity, with stronger effects in schizophrenia than bipolar disorder, 3) sub-thalamic nuclei show differential effect of group on cortical connectivity, with more pronounced alterations in patients in frontal-projecting nuclei, and 4) decreased thalamic volume and shape alterations in patients.

Results revealed decreased within-thalamic connectivity, particularly in frontal-projecting regions. Network-based analysis revealed reduced negative correlation between the thalamus and the left frontoparietal component in schizophrenia patients, while bipolar disorder patients exhibited an increased positive correlation with a somatomotor component. Sub-regional analysis revealed patterns of reduced thalamic connectivity with frontal and posterior cortical regions and increased with sensory regions in schizophrenia. Connectivity was reduced within all sub-thalamic seeds in schizophrenia, and to a lesser extend in three seeds in bipolar patients. Moreover, reduced gray matter and shape abnormalities were found in frontal-projecting regions in both schizophrenia and bipolar disorder, but did not seem to explain reduced functional connectivity. Thus, all imaging modalities converged on disrupted fronto-thalamic connectivity, with strongest reductions in schizophrenia, in addition to increased somatosensory-thalamic coupling. These findings support that thalamo-cortical interactions are crucial for optimal brain function, and provide further evidence for a role of thalamo-cortical interactions in the pathophysiology of psychotic disorders.

3.3 Paper III

Schizophrenia is a severe mental illness with high heritability and complex etiology. However, previous neuroimaging findings are inconsistent, likely due to a combination of methodological and clinical variability and relatively small sample sizes. Few studies have used a data-driven approach for characterizing pathological interactions between regions in the whole brain and evaluated the generalizability across independent samples. To overcome this issue, we performed a comprehensive brain imaging investigation of functional brain connectivity in a large cohort of schizophrenia patients and healthy controls from three different samples, numbering a total of 530 subjects. Thus, the aim of our third study was to
identify altered interactions between brain networks in schizophrenia, to assess the reliability of the effects across three independent samples, and to test to what degree these findings could be used to correctly classify patients and controls. We hypothesized an overall reduced connectivity in schizophrenia patients, particularly encompassing frontal, sensory and perceptual nodes. Moreover, in line with the conception that fMRI-based functional brain connectivity is a sensitive and robust intermediate phenotype for psychotic disorders, we expected that the multivariate classifier trained on one sample would perform reasonably well in the independent datasets.

Data-driven definitions of nodes in combination with regularized partial temporal correlation analysis revealed reduced connectivity in schizophrenia in frontal, sensory, and subcortical networks, which was consistent in all three samples. Moreover, training a multivariate classifier on the TOP sample resulted in an accuracy up to 80% in the combined HUBIN+KASP sample, supporting that the clinical sensitivity of the brain network connectivity measures is generalizable across samples and scanners. Effect of medication, symptom scores, duration of illness, diagnostic subgroups, subject motion, and tSNR were negligible and had very limited effect on the identified group differences.
4. DISCUSSION

4.1 Summary of results

To summarize, the main findings of the present thesis are:

1) Reduced centrality in limbic structures in schizophrenia and bipolar disorder, and somatosensory and visual regions in schizophrenia.
2) Increased centrality of regions overlapping with the DMN in schizophrenia, with a graded response in bipolar disorder.
3) Reduced thalamo-frontal coupling and increased thalamic connectivity with sensory regions in schizophrenia. Increased thalamo-somatomotor coupling in bipolar disorder.
4) Reduced connectivity between several networks in schizophrenia, inducing frontal, sensory, and subcortical regions, with consistent findings across independent samples.

In the following section, the results from the studies included in this thesis will be discussed in relation to previous findings and organized according to brain regions. Methodological issues will then be discussed, before conclusions and comments on future directions.

4.2 Functional brain alterations

4.2.1 Sensory dysfunction

Schizophrenia has been conceptualized as a cognitive disorder, and therefore much attention has been given to higher order cognition (such as working memory) or the role of the DMN. Sensory processing, on the other hand, has received much less attention in the neuroimaging field. Interestingly, sensory regions were affected in patients in all three studies, regardless of study approach or methodology. In schizophrenia, we found visual, somatomotor, and auditory regions to be affected. Bipolar disorder had no centrality changes in these regions.

The visual cortex was affected in schizophrenia in papers I and III. Five visual nodes showed reduced correlation with other visual nodes (paper III). Three of these nodes represented early visual cortices, two of which had reduced connectivity with V4 and one that had reduced connectivity with V5. The ventral stream goes through V4 on to the inferior temporal cortex, and is critical for object recognition, visual perception and memory, and the dorsal stream passes through V5 (visual motion). In paper I, the voxel-wise eigenvector centrality analysis showed a decreased centrality in left and right occipital cortex, overlapping with V4 and V5. Decreased centrality and connectivity in visual regions suggests
less coordinated sensory processing, which may be related to the range of cognitive and emotional symptoms through various downstream mechanisms (Javitt & Freedman, 2015; Revheim et al., 2014). Jointly, these findings demonstrate that both major paths of visual processing are affected, which further strengthen the conception of dysfunctional multi-level visual information processing in schizophrenia (Butler et al., 2008; Javitt, 2009).

Three nodes within the somatomotor cortex showed both reduced connectivity (paper III) and reduced eigenvector centrality (paper I) in schizophrenia. The somatomotor system has previously been implicated in schizophrenia using different approaches (Berman et al., 2016; Butler et al., 2008; Javitt, 2009; Kaufmann et al., 2015). Moreover, the auditory node showed reduced connectivity with bilateral temporal and right frontal regions (paper III). Basic auditory processing impairments have been documented in schizophrenia (Javitt & Sweet, 2015), and auditory hallucinations is one of the core symptoms (Hugdahl, 2009). Disrupted flow of information between the auditory cortex and other regions may indicate an underlying neurobiological pathology in patients.

Bipolar disorder patients did not show the same deficiency in sensory regions as schizophrenia. In paper I, schizophrenia had decreased occipital and somatosensory centrality compared to bipolar disorder, suggesting stronger implications of sensory and visual circuits in schizophrenia. However, since bipolar disorder patients were not included in study III, we cannot rule out any altered correlations between or within sensory regions. Together, these findings support that sensory processing deficits are a hallmark of schizophrenia, and that different methodological approaches point to dysfunction in the same regions.

4.2.2 Limbic regions

Paper I showed reduced centrality in hippocampus/amygdala and putamen in schizophrenia and bipolar disorder. These are limbic regions involved in processing and regulation of memory functions and emotions, shown to be implicated in both schizophrenia (Heckers & Konradi, 2010; Rimol et al., 2010) and bipolar disorder (Frey et al., 2007; Garrett & Chang, 2008). The main feature of bipolar disorder is alternate episodes of mania and depression. The amygdala has been shown to have altered cortical connectivity in these two phases in bipolar disorder patients, indicating its role in regulating different mood states (Cerullo et al., 2012; Garrett & Chang, 2008). Reduced centrality in these regions highlights a disruption in mood circuits, which are characteristic features of both disorders.
The putamen makes up part of the striatum, believed to be involved in generating cognitive symptoms in schizophrenia (Simpson et al., 2010). The putamen has many dopamine receptors, and is therefore one of the main targets of antipsychotic medication. One study showed that the putamen increased in size after treating drug-naïve schizophrenia patients with AP for 6 weeks, and that this was associated with a decrease in positive symptoms (Li et al., 2012). Centrality was reduced in patients, and in paper III we observed decreased connectivity between the putamen and two premotor nodes. As the basal ganglia motor circuit involves the thalamus, putamen, and premotor cortex (DeLong & Wichmann, 2007), altered connectivity of these nodes may indicate poorer functioning of cortico-basal ganglia pathways and subsequent reduced motor function (Bracht et al., 2013).

4.2.3 Reduction of hubs in sensory and limbic structures

In paper I, we found that all clusters with decreased centrality (sensory and limbic structures) had a high average centrality compared to whole brain mean, supporting the notion of reductions of hub-regions in patients (Crossley et al., 2014; Rubinov & Bullmore, 2013; van den Heuvel & Fornito, 2014). Hubs are essential for efficient information flow and integration and more likely to be symptomatic if affected (Crossley et al., 2014; van den Heuvel & Sporns, 2013). Likewise, being more biologically costly to maintain makes hubs vulnerable to pathology (Crossley et al., 2014; van den Heuvel & Sporns, 2013).

Although high centrality clusters were decreased, not all high centrality regions in the brain were affected, suggesting specific targeting of hub-regions in schizophrenia and bipolar disorder. Clearly, our results indicate some specificity of targeted hubs; high centrality limbic structures were reduced in both schizophrenia and bipolar disorder, while somatomotor and visual regions were specific to schizophrenia patients. The question pertaining to clinical specificity is particularly relevant in light of recent work implicating high-centrality regions in a range of disorders (Crossley et al., 2014), demonstrating that perturbations to hub regions are more likely to be symptomatic than alterations in non-hub regions.

4.2.4 The role of the thalamus

In paper II, the whole-brain voxel-wise analysis in the thalamic component showed a reduced connectivity in schizophrenia bilaterally in two thalamic clusters, overlapping with prefrontal-projecting sub-regions. The whole-brain network-based analysis showed that schizophrenia patients had reduced thalamic coupling with a left frontoparietal component,
including frontal, parietal, and temporal regions, all of which have previously been implicated in schizophrenia (Fornito et al., 2012; Karbasforoushan & Woodward, 2012). We found no effects in bipolar disorder, suggesting a specific pattern for schizophrenia. We observed increased thalamic-somatomotor coupling in bipolar disorder, which is in line with a previous report (Anticevic et al., 2014a), but not in schizophrenia. Increased thalamic connectivity with somatomotor regions has been observed in several schizophrenia studies (Anticevic et al., 2014a; Kaufmann et al., 2015; Pergola et al., 2015; Woodward et al., 2012), indicating increased information flow to the somatomotor cortex. In paper III, we extended these findings by documenting reduced thalamo-temporal connectivity in all three samples.

The thalamus is highly heterogeneous, and its nuclei are largely segregated with differential cortical and subcortical projections. Discrepancies observed when studying the thalamus as a whole are likely to stem from pathophysiological processes in distinct nuclei (Pergola et al., 2015). Previous studies have divided the cortex into large regions of interest (Woodward et al., 2012), which provide useful information about specific patterns of thalamo-cortical projections. However, assessing correlations voxel-wise across the brain provides more detailed information (Anticevic et al., 2014a), in particular considering that thalamo-cortical connectivity patterns, much like resting-state networks, may span several lobes (Yuan et al., 2015).

Our findings indicate differential connectivity alterations in the patient groups for the ten sub-thalamic seeds, though many similarities were found. Particularly, reduced within-thalamic connectivity was found for all seeds in schizophrenia, mimicking the whole-thalamus analysis, and three seeds in bipolar disorder. Eight seeds showed reduced frontal connectivity in schizophrenia, most of which also extended to posterior brain regions, in line with our findings of reduced thalamo-frontoparietal connectivity. In fact, decreased connectivity with frontal lobe is one of the main thalamo-cortical disruptions observed in schizophrenia (Anticevic et al., 2014a; Cheng et al., 2015; Wang et al., 2015; Woodward et al., 2012), pointing to the role of thalamus in the regulation of cortical regions involved in cognitive functions.

Two seeds also showed increased connectivity with sensory regions in schizophrenia, including somatomotor, visual and auditory cortices, in line with previous studies (Cetin et al., 2014; Wang et al., 2015). Again, these are the same sensory regions also implicated by reduced centrality (paper I) and reduced partial correlations (paper III). Based on the influential sensory gating hypothesis (Cromwell et al., 2008), altered thalamo-cortical connectivity and information flow to sensory regions could reflect poorer filtering
mechanisms in the continuous extraction of relevant stimuli, and the characteristic psychotic, cognitive and perceptual distortions.

Structural studies on the thalamus have provided inconsistent results, though the main consensus points to reduced volume both in schizophrenia (Byne et al., 2009; Pergola et al., 2015; van Erp et al., 2015) and bipolar disorder (Hibar et al., 2016). We identified focal thalamic gray matter reductions and medial shape alterations in schizophrenia and bipolar disorder, in regions largely projecting to frontal and temporal cortices (Behrens et al., 2003). Thalamic gray matter reductions in schizophrenia are often reported (Pergola et al., 2015), with post-mortem findings pointing to greatest neuronal loss in the mediodorsal nucleus (projecting to frontal cortex) and pulvinar (linked to visual attention), again pointing to disrupted communication flow to frontal and sensory regions (Byne et al., 2009; Fischer & Whitney, 2012; Pergola et al., 2015).

Similar to our findings, other studies have also found shape abnormalities in medial thalamus in schizophrenia (Smith et al., 2011a; Thong et al., 2013), in schizophrenia and their non-psychotic siblings (Harms et al., 2007), and in antipsychotics-naïve schizophrenia patients (Danivas et al., 2013). Structural alterations in frontal- and temporal-projecting thalamic regions in schizophrenia (Pergola et al., 2015) implicate the communication flow between the thalamus and these cortical regions in the pathophysiology.

Importantly, functional connectivity alterations in psychotic disorders may partly stem from structural brain differences. However, whereas shape and VBM analysis converged on the medial parts of the thalamus in schizophrenia and bipolar disorder, effects were moderate (only significant in direct comparisons between the groups). Also, although reduced VBM was moderately associated with reduced connectivity, adding VBM as a covariate did not weaken the group effects on functional connectivity, indicating complex relationships between structural and functional alterations. In summary, structural changes converged on frontal-projecting thalamic regions, but effects were moderate and are unlikely to explain the altered thalamic functional connectivity.

4.2.5 Higher order cognitive regions
The frontal lobe is critical for cognitive functions such as planning and problem solving, and has been shown to exhibit reduced connectivity in schizophrenia (Fornito et al., 2012; Pettersson-Yeo et al., 2011). In paper III, several of the edges showing group differences involved frontal lobe nodes, two of which also showed a strong cumulative effect of
diagnosis also beyond the edges surviving strict correction for multiple comparisons. Differential frontal connectivity may reflect reduced executive functioning, planning, and abstract thinking in schizophrenia.

The DMN is a widely studied network, often shown to be altered in schizophrenia (Broyd et al., 2009; Whitfield-Gabrieli & Ford, 2012). In paper I, increased centrality in schizophrenia was found in frontal and posterior parietal lobes, largely overlapping with DMN regions, while paper III identified reduced connectivity between a medial frontal node and the precuneus, also part of the DMN. These regions were also implicated in many edges in schizophrenia, indicating that default mode network nodes are critical in the pathophysiology, and that DMN-associated introspection and self-referential may be altered (van den Heuvel & Fornito, 2014; Whitfield-Gabrieli & Ford, 2012).

In paper I, bipolar disorder showed intermediate centrality in frontal and posterior parietal clusters, exhibiting significant increases compared to healthy controls in left parietal and right frontal lobe, and decreases compared to schizophrenia in frontal clusters and right parietal lobe. Findings of intermediate eigenvector centrality in bipolar disorder patients in these clusters suggest that the patient groups may indeed reflect different parts along the same continuum (Argyelan et al., 2014; Brandt et al., 2014).

This pattern of a relative decrease in the centrality of select hub-like regions and an increase in low-centrality clusters suggests a smaller range in centrality values in schizophrenia and bipolar disorder, as seen in other studies (Lynall et al., 2010), indicating less functional specialization or brain network differentiation. A distribution of low centrality regions with some high degree hubs has been shown to be an optimal network configuration (van den Heuvel & Sporns, 2013), and a more uniform distribution of eigenvector centrality in patients would be indicative of a less efficient network.

4.2.6 Reliability and clinical classification

In paper III, the group effects for the 14 significant edges were in the same direction in all three samples with comparable effect sizes across edges, supporting the robustness of the findings. Six and eight of the effects were replicated within KASP and HUBIN, respectively, likely reflecting the smaller sample sizes, which may also explain some of the discrepancies in the literature (Pettersson-Yeo et al., 2011). Also, nominal effect of diagnostic subgroup was restricted to only two of the significant edges, suggesting common functional brain pathology across the psychosis spectrum.
Using a multivariate machine learning approach utilizing the full set of edges we found a high overall accuracy in classifying patients and controls. Training the classifier on TOP gave good accuracies in the combined KASP+HUBIN sample, and even in the smaller individual samples. Training the classifier on KASP+HUBIN gave slightly lower accuracy in TOP, likely due to a smaller sample size. These robust validations are important since they suggest generalizability of connectivity aberrations across different scanners and heterogeneous samples, including both recently diagnosed and chronic patients. Consequently, our results suggest that a mega-analysis attempt similar to those recently deployed in the structural imaging domain (Hibar et al., 2015; van Erp et al., 2015) and genetics (Thompson et al., 2014) may well be feasible for functional connectivity analysis and may be key to shed light into biologically informed clinical subgroups in our global efforts toward developing a precision medicine approach in psychiatry (Insel & Cuthbert, 2015).

4.2.7 Clinical associations
The association with clinical variables within patient groups was in general non-existent or moderate in all three studies. In paper I, within-group associations showed no significant associations between symptom scores and centrality in either patient group, both for the 12 clusters with effect of diagnosis and the whole-brain voxel-wise analyses. However, some trends were seen and further studies are needed to assess their reliability and clinical significance. In paper II, however, the voxel-wise analysis in the thalamic component revealed a significant effect of group on connectivity bilaterally in two thalamic clusters. The mean connectivity of these two clusters showed significant associations with depressive symptoms in bipolar disorder and general psychopathology in schizophrenia, indicating decreasing connectivity with increasing symptom burden. Thalamic connectivity has previously been associated with clinical severity (Anticevic et al., 2014a; Cheng et al., 2015), supporting some degree of state dependency. In paper III, none of the edges were associated with total PANSS scores or duration of illness.

4.2.8 Implications for pathophysiology
The findings of the current thesis support the dysconnectivity hypothesis (Friston, 1998; Stephan et al., 2009) of schizophrenia, implicating aberrant connectivity between distinct regions in the brain. Altered functional connectivity was found in patients across all three
studies in the whole brain, even when using different approaches and methodologies, supporting a global disintegration of connections in the brain. These findings are of importance as they show a disrupted information flow in patients group, particularly in schizophrenia, with a generally poorer communication between most brain regions, though with some regions showing hyperconnectivity.

Interestingly, our findings highlight the importance of brain regions involving sensory processing in the pathophysiology of schizophrenia. If the sensory input is already faulty before being passed on to higher level processes involving the frontal lobe, in which the thalamus plays a central role, it could partially explain the cognitive difficulties observed in schizophrenia. As of today, the main focus of neuroimaging studies has been on the DMN and cognitive processes, such as working memory, while current results suggest more attention should also be given to characterize early sensory processing deficits.

One hypothesis that has received much attention in recent years is the continuum hypothesis of psychotic disorders (Craddock & Owen, 2010; van Os et al., 2009). In this regards, psychosis should be regarded as a continuum with gradual transitions from one diagnosis to another, as opposed to separate entities. Some regions, such as frontal, parietal, and some subcortical structures did show bipolar disorder to be intermediary between schizophrenia and healthy controls. However, although we only included bipolar disorder patients in paper I and II, sensory regions seems to be specific for schizophrenia, whereas limbic regions were equally affected in both groups. Our findings thus only partially support the continuum hypothesis, and additionally suggest distinct characteristics for schizophrenia (sensory regions and thalamo-frontal dysconnectivity) compared to bipolar disorder, and common features equally affected in both patient groups (limbic regions). Indeed, the continuum hypothesis has been critiqued by some as being “scientifically unproven and clinically impractical” (Lawrie et al., 2010), and may therefore not fully capture the underlying pathophysiological processes in psychosis.

4.3 Methodological considerations

4.3.1 Study sample

There are several factors to consider with regards to our study sample. These include the recruitment of participants and their demographics, sample size and diagnostic subgroups, and effects of medication.
Demographics

In our samples, there was some bias in the selection of both patients and healthy controls. The TOP study includes primarily recently diagnosed patients (within 5 years). Both in- and out-patients were recruited with varying disease severity, though none in the acute illness phase, indicating that our patient sample may not represent the entire schizophrenia population. In study III, two additional patient samples were included with a different clinical composition. KASP patients were first episode, recently diagnosed young patients, many of whom were antipsychotics-naïve, while HUBIN patients were older, chronic patients from a 12-year follow-up. Our three patient samples are therefore very different, adding to the variability in the final study.

Controls in the TOP study were recruited by sending letters of invitation to a randomly drawn sample from the Norwegian national register. However, there is likely to be some bias in which individuals decided to participate, such as having a higher education, better social functioning, and interest towards scientific research. The control sample might therefore not be fully representative of the general population.

Age and sex are important confounders on brain activation. Brain structure changes throughout the lifespan (Fjell & Walhovd, 2010), and differences have also been observed between males versus females (Gong et al., 2011). To control for the effects of these factors, we added age and sex as covariates in all our analyses, and recruited controls within the same age group so as to best match patients and controls at the group level. Subject groups differed on IQ and education, which is common in clinical studies, and may reflect both recruitment biases and common mechanisms related both to the disease and the relevant phenotype. The heterogeneity of the subject samples is likely to contribute to some of the variance observed in the current studies.

Sample size and subgroups

One major strength of our studies is that we included a relatively large group of patients and controls. Psychiatric diagnoses are heterogeneous, and the increased statistical power of using a large sample makes it possible to detect subtle effects. For this reason, we chose to include a larger diagnostic spectrum not only limited to a strict schizophrenia diagnosis, and rather perform post hoc analyses to test if the subgroups behaved differently. Diagnostic categories of bipolar disorder and schizophrenia are still based on a clinical interview assessing the symptomatology, and the lack of biomarkers in setting a diagnosis could be an underlying
contributor to the heterogeneity found in psychiatric patient groups. Still, patients within the psychosis spectrum are likely to share an underlying disease pathophysiology.

Post hoc analyses revealed few effects of subgroups. Paper I showed no centrality differences between the subgroups in the identified clusters. In paper II, effects of reduced thalamic functional connectivity remained (though weakened slightly) when excluding the schizophrenia spectrum sub-groups, indicating the effect was not only present in schizophrenia. Subgroups differed within the bipolar group, however, where the increased thalamo-somatmotor correlation was driven by the bipolar disorder type I patients (paper II). In paper III, nominal effect of subgroup was only found in two of the edges showing main effect of diagnosis.

**Medication**

One limitation is that many patients were medicated, and as such the patient-control differences could be partially due to medication effects on the brain. However, caution is needed when assessing effects of medication and cognitive functions as they are difficult to disentangle using the current study design because of the inherent collinearity between medication type, dose, diagnosis and clinical variables. Ideally, this should be assessed in a properly designed randomized controlled trial.

However, we did investigate if antipsychotic medication dose was associated with any of the main findings. In paper I we tested for centrality differences between bipolar disorder patients with and without antipsychotics, which revealed no differences. Centrality differences between schizophrenia and bipolar disorder patients on antipsychotics showed similar effects as when also including the unmedicated patients. In paper II, antipsychotic daily dose was not associated with altered thalamic functional connectivity in schizophrenia or bipolar disorder I patients, while in paper III effects of group were also found in the KASP sample where about half of the patients were not medicated. Moreover, the one edge that showed an effect of medication in paper III was in the opposite direction of the patient-control difference, making it unlikely that our findings are purely a result of medication effects. Together, these findings support the notion that effects observed in the current thesis are not purely a result of medication status.
4.3.2 fMRI acquisition

The BOLD-signal is an indirect measure of neuronal activation. fMRI allows for measuring activation patterns across time \textit{in vivo}, but the method is not without limitations. First, the detection of changes in brain blood flow is very small (around 5\%) in comparison to the signal in the rest of the brain (Huettel et al., 2004). Also, as magnetic field strength increases, some regions become harder to measure due to signal losses near air-tissue boundaries. Regions with signal drop-out, particularly the orbitofrontal cortex and parts of the temporal lobe, were therefore not included in the analysis and we cannot make any inference of group effects in these regions. Moreover, due to restricted field of view, parts of the cerebellum were also excluded.

The BOLD-signal is also subject to different noise factors, which may mask the neuronal signal. These include physiological factors (pulse and respiration), thermal noise, and system noise from the scanner hardware (such as inhomogeneities in the magnetic field) (Huettel et al., 2004). Artifacts related to in-scanner subject motion reflect a challenge in neuroimaging, in particular when targeting the covariance structure of the time series. In general, patients moved more than controls, which may be a potential confounder. To limit the effect of motion, we used FIX to remove artifacts related to head motion and other sources of noise for each individual. So, although patients had more head motion they also had more noise components removed, suggesting a slightly more aggressive cleaning in these datasets.

As any residual effects of motion might remain, we also added motion as an additional covariate to some analyses. In paper I, adding motion had no effects on the group differences in EC. In paper II, motion was not associated with the significant effects on functional connectivity in schizophrenia or bipolar disorder type I, while in paper III, the main effects of group on edgewise connectivity remained after adding motion as a covariate.

Due to the influence of the different noise factors on the BOLD-signal, acquisition sequences and processing steps are continuously developed to increase the SNR. In our studies, running FIX also strongly increased the temporal SNR of the data in both patients and healthy controls, though patients had lower tSNR than controls both before and after running FIX. Although some edges were associated with tSNR in controls in paper III, adding tSNR to the main model as a covariate did not remove any of the significant group effects, and is therefore unlikely to explain the group differences.
4.3.3 Analytical approaches

**MRI processing**
The choice of preprocessing and analysis approach of brain images can affect the findings (Churchill et al., 2012). The pipeline for preprocessing imaging data involves many steps, each of which has numerous options. The choice of the analysis approach and statistical analyses is also abundant. Thus, the diversity in methodological approaches is likely one of the main contributors for different findings across studies, and, although we used standard accepted methods for processing the data, there is no “correct” way of doing this. Moreover, as advances in methodology improve, the accepted norm today is likely to change in the years to come. Thus, it is important to be aware of how each step in the pipeline affects the data, and to understand the limitations of fMRI analysis.

**Eigenvector centrality mapping (ECM)**
Centrality measures probe the relative importance of and identify brain regions exhibiting hub-like properties (Joyce et al., 2010; Zuo et al., 2012), which reflect critical modules in the processing systems of the brain. Although ECM has only been used in a few clinical studies so far (and none in schizophrenia), the eigenvector centrality (EC) map across all subjects showed a network of high-centrality regions similar to other ECM studies, supporting the reliability of the method (Binnewijzend et al., 2014; Lohmann et al., 2010; Schoonheim et al., 2014).

Relying on measures of full correlations when assessing the structure of the brain network, which is the case for the current implementation of ECM (Lohmann et al., 2001), may be less reliable and more vulnerable to noise than estimates of partial correlations (Smith et al., 2011b). However, using partial correlations to estimate EC on a whole-brain sized matrix is currently computational infeasible, but further studies utilizing alternative definitions of the connectivity matrices are needed.

**Independent component analysis (ICA)**
As mentioned in the introduction, there are several ways to define and describe brain networks. However, great caution is needed in defining the network nodes, such as by using anatomical brain atlases, as the consequent network estimation are very vulnerable to inappropriate definitions (Smith et al., 2011b). The use of group ICA to define the nodes in the brain network enabled us to overcome some of the limitations related to atlas-based approaches, as the definition of nodes is data-driven (Smith et al., 2011b; Smith et al., 2013).
However, the number of components can be adjusted, usually ranging from 20 to 80. At a lower decomposition, several regions are more likely to be grouped together into one network, whereas at a higher decomposition the same regions might constitute separate networks. In this sense, the ICA-approach is not fully data-driven, and there is some bias as to which decomposition the researcher deems to be optimal.

**Partial vs. full correlations**

In paper II and III, we used partial regularized correlations, with the aim to estimate direct connection strengths with higher accuracy than is achieved when using full correlations (Marrelec et al., 2006). Full correlations measure the extent of temporal association between two components’ time series, while partial correlations additionally control for the dependencies on common influences from other components (Brier et al., 2015; Marrelec et al., 2006). Removing the dependencies between two components on other regions may make partial correlations more suitable to study direct functional interactions between pairs of nodes (Marrelec et al., 2006). Most previous network-level studies have considered only one or a small number of nodes, usually targeting simple full correlations between components. It is therefore unknown whether connectivity between two nodes could be due to shared variance with a third node. Partial correlations may provide a more “direct” measure, while also, to a much larger extent than full correlations, correcting for non-neural physiological noise (Fox et al., 2009; Van Dijk et al., 2010).

**Multiple comparisons**

An important aspect of statistical tests is correction for multiple comparisons. This is necessary to avoid type I errors, meaning detecting a group difference that is not present (false positive). Increasing the number of tests also increases the likelihood of finding a difference, and thus the most common way of dealing with multiple testing is Bonferroni-correction. In this case, the critical p-value (0.05) is divided by the total number of tests, setting a new threshold for significance. We have generally used this strict threshold in our statistical analyses, except at the voxel-level which require a different approach. One challenge in fMRI is the immense number of statistical tests, usually involving several thousand voxels. In this case, Bonferroni-correction will be too strict, and alternate methods are needed. In all three papers, we performed permutation testing for all voxel-based analyses, which obtains the sampling distribution of the test statistic by rearranging the subject labels and running several permutations. We then corrected for multiple testing using
threshold free cluster enhancement (Smith & Nichols, 2009), where groups of voxels are considered more likely to be true differences than single voxels.
5. CONCLUSIONS AND FUTURE DIRECTIONS

We have demonstrated altered functional brain connectivity in patients with schizophrenia and bipolar disorder, with more substantial changes in schizophrenia patients. Modalities from centrality measures, voxel-wise connectivity in specific regions and between-network correlations all converge on altered connectivity in sensory, frontal, limbic, and thalamic regions in patients. All studies showed reduced centrality and connectivity involving sensory nodes (visual, somatomotor, and auditory regions) in schizophrenia, suggesting the cognitive deficits observed in these patients may be partially explained by aberrant sensory processing. The frontal cortex also exhibited altered connectivity, in line with the observed cognitive difficulties in patients, while reduced limbic connectivity highlights deficiencies in the mood-circuits of the brain in both patient groups.

From our second paper, it seems likely that the thalamus plays an important role in this observed dysconnectivity in patients. In schizophrenia, both functional and structural modalities indicated disrupted thalamic communication flow to the frontal lobe, likely affecting higher-order cognitive processing, and increased connectivity to sensory regions, highlighting the potential role of the thalamus in manifesting sensory disruptions in psychosis. Moreover, the connectivity changes in paper III were robust across two scan sites, with good classification accuracies in the independent samples, pointing to common brain alterations in schizophrenia. Our findings show that dysregulated brain functional connectivity is evident in both schizophrenia and, to a lesser extent, bipolar disorder and that the characteristic perceptual and cognitive distortions in these patient groups may be partly explained by information processing deficits between sensory, limbic, thalamic, and frontal regions.

Future research

There are several future studies that would be of interest to pursue based on the current findings, with regards to both methodological and clinical factors. First of all, there is a need for replication in independent samples, particularly in the first paper as this was a new method not previously used in schizophrenia and bipolar disorder patients. In the current thesis, we primarily looked at functional connectivity. However, by the use of multimodal imaging, the relationship with underlying gray and white matter integrity could be evaluated. Also, imaging genetic studies could be performed to evaluate the association between altered connectivity and vulnerability genes.
With regards to clinical factors, the findings of the third paper should be extended to include bipolar disorder patients to further characterize the specificity of the altered functional connectivity. Moreover, since sensory regions were clearly implicated in schizophrenia, future studies specifically targeting these modalities should be conducted to gain further knowledge of sensory processing deficits in these patients. Ideally, studies should be extended to antipsychotics naïve patients to fully rule out medication effects, and to include siblings of the patients to test for the effect of overlapping genes. At risk patients likely to develop a psychotic disorder could also be included in a longitudinal study, to see if there are any predictive baseline differences in brain connectivity in the individuals who eventually develop schizophrenia.

To conclude, the current thesis provides new knowledge about functional resting state brain connectivity in schizophrenia and bipolar disorder, pointing to underlying disease mechanisms in these disorders. Hopefully, with an increasing endeavor and a growing number of studies, further insights and knowledge will improve diagnostics, treatment, and quality of life for patients with these devastating disorders.
6. REFERENCES


in fMRI are related to cognition and CSF biomarkers. *Hum Brain Mapp.*, 35(5), 2383-2393.


PAPERS I-III