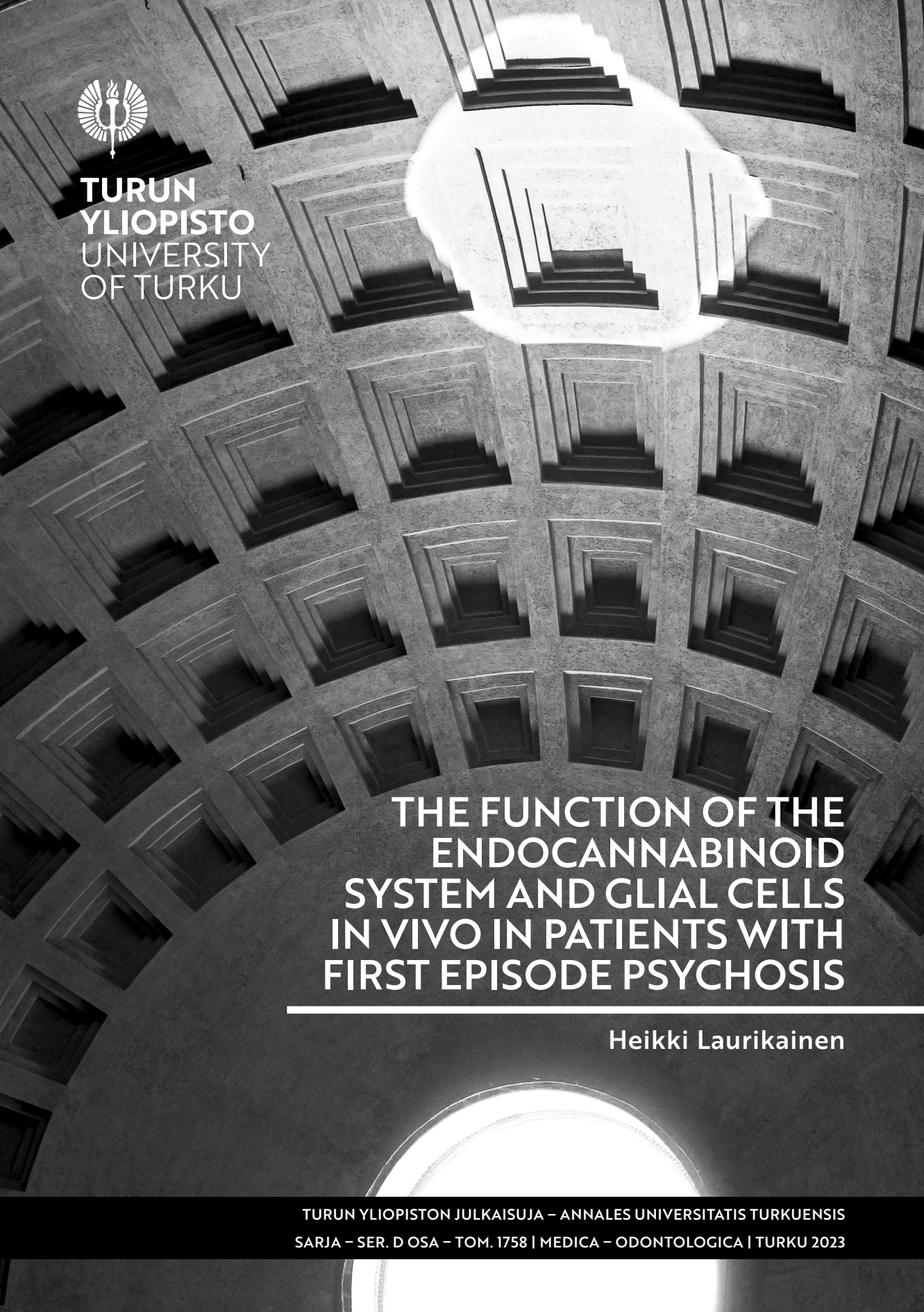

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THE FUNCTION OF THE ENDOCANNABINOID SYSTEM AND GLIAL CELLS IN VIVO IN PATIENTS WITH FIRST EPISODE PSYCHOSIS

Heikki Laurikainen

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To children, and the children within:

UNIVERSITY OF TURKU
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ABSTRACT

Psychoses are relatively common and often severely debilitating mental disorders with a multifactorial etiological background involving both psychosocial and biological factors. Previously reported associations between the endocannabinoid and immune systems, and psychotic disorders, suggest that they are involved in the etiology of psychosis.

Healthy individuals were studied with the selective type 1 endocannabinoid receptor (CB1R) radiotracer [^{18}F]FMPEP-*d2,* and positron emission tomography (PET), for possible demographic confounders. Radiotracer synthesis and the compound's behaviour in blood and brain tissues, were in line with reports from previous validation studies. Females had lower availabilities of CB1R than males in 17 discrete brain regions.

Separate samples of male patients with first-episode psychosis (FEP) were then studied concurrently in Turku and London, using the CB1R radiotracers [^{18}F]FMPEP-*d2* and [^{11}C]MEPPEP respectively. Lower CB1R availability was seen in FEP as compared to healthy controls. The availability of CB1R was also inversely associated with the symptomatology of the psychoses.

Translocator protein (TSPO) expression has been postulated to represent glial cell and mitochondrial functions, both of which are influenced by endocannabinoid signalling. Another sample of male and female patients with first episode psychoses was studied using PET with the selective TSPO radiotracer [^{11}C]PBR28. Male and female FEP subjects showed globally lower availability of brain TSPO in comparison to healthy controls. Two concurrent samples of FEP individuals showed persistent elevations of the chemokine CCL22 when compared to population controls. A subgroup of patients with the highest levels of CCL22 also had aberrant levels of other cyto- and chemokines.

These results indicate that the immune and brain endocannabinoid systems have become dysregulated in early psychosis. Aberrant glial cell function and/or disturbances in cell metabolism are indicated by the lower availability of TSPO.

KEYWORDS: psychosis, schizophrenia, psychotic mood disorder, endocannabinoid system, type 1 endocannabinoid receptor, translocator protein, positron emission tomography, immune signalling, microglia, astrocyte

TURUN YLIOPISTO
Lääketieteellinen tiedekunta
Kliininen laitos
Psykiatria
HEIKKI LAURIKAINEN: Endokannabinoidijärjestelmän ja gliasolujen toiminta ensipsykooseissa
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TIIVISTELMÄ

Psykoosit ovat verrattain yleisiä, vakavia mielenterveyshäiriöitä, joiden syntyyn vaikuttaa sekä psykososiaaliset että biologiset tekijät. Endokannabinoidi- ja immuunijärjestelmien yhteydet psykooseihin, sekä dopamiinijärjestelmän toimintaan, viittaavat näiden järjestelmien toimivan osana psykoosien etiologiaa.

Terveiden koehenkilöiden aivojen endokannabinoidijärjestelmän toimintaa tutkittiin tyypin 1 endokannabinoidireseptorin (CB1R) merkkiaineella [^{18}F]FMPEP-*d2,* ja positroniemissiotomografialla (PET), mahdollisten sekoittavien tekijöiden tunnistamiseksi. Merkkiaineen tuotannon laatua kuvaavat tunnusluvut, sekä merkkiaineen käyttäytyminen veressä ja aivokudoksessa, vastasivat aiempien validointitutkimusten tuloksia. Naiskoehenkilöillä oli alhaisemmat [^{18}F]FMPEP-*d2*:n jakautumistilavuudet 17 aivoalueella verrattuna miehiin.

Miespuolisten ensipsykoosipotilaiden aivojen endokannabinoidijärjestelmän toimintaa tutkittiin erikseen Turussa ja Lontoossa PET:lla vastaavasti CB1R merkkiaineilla [^{18}F]FMPEP-*d2* ja [^{11}C]MEPPEP. Molempien otosten ensipsykoosipotilailla oli alhaisemmat merkkiaineiden jakautumistilavuudet verrattuna terveisiin koehenkilöihin. Merkkiaineen sitoutumiselle vapaat CB1R:t olivat lisäksi käänteisesti yhteydessä psykoosioireiden vaikeusasteeseen.

Aivojen tukisolujen ja näiden mitokondrioiden toimintaan vaikuttavat sekä endokannabinoidiviestintä, että translokaattoriproteiinin (TSPO) toiminta. Ensipsykoosipotilailla oli kauttaaltaan alhaisemmat TSPO PET merkkiaineen [^{11}C]PBR28 jakautumistilavuudet verrattuna terveisiin verrokkihenkilöihin. Ensipsykoosipotilaiden kemokiini CCL22:n pitoisuudet olivat verrokkien pitoisuuksia korkeammat. Korkeimpia CCL22:n pitoisuuksia omaavien potilaiden immuuniviestintä poikkesi muista verrokki- ja potilastutkittavista laaja-alaisesti.

Nämä tulokset osoittavat, että immuuni- ja endokannabinoidijärjestelmät toimivat poikkeavasti ensipsykooseissa. TSPO:n poikkeava toiminta viittaa siihen, että aivojen tukisolut ja/tai solujen aineenvaihdunta häiriintyvät psykooseissa.

AVAINSANAT: psykoosi, skitsofrenia, affektipsykoosi, endokannabinoidijärjestelmä, tyypin 1 endokannabinoidireseptori, translokaattoriproteiini, positroniemissiotomografia, immuuniviestintä, mikroglia, astrosyytti

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Abbreviations

^{11}C	Carbon-11
^{18}F	Fluoride-18
2-AG	2-arachinodoylglycerol
2TCM	2-tissue compartment modelling
AA	Arachidonic acid
ABHD6	Alpha/beta-hydrolase containing 6
AEA	anandamide
ATP	Adenosine triphosphate
BOLD	Blood-oxygen-level-dependent
C3	Complement factor 3
C4	Complement factor 4
Ca2+	Calcium-ion
CB1R	Endocannabinoid receptor type 1
CB2R	Endocannabinoid receptor type 2
CCK+	Cholecystokinin positive
CCL	C-C motif chemokine
CCR	C-C motif chemokine receptor
CNS	Central nervous system
CNV	Copy number variant
DAG	Diacylglycerol
DAGLα	Diacylglycerol lipase alpha
DMN	Default mode network
DSE	Depolarization induced suppression of excitation
DSI	Depolarization induced suppression of inhibition
DSM	Diagnostic and statistical manual
ECS	Endocannabinoid system
EEG	Electroencephalography
E-I	Excitation-inhibition
CEN	Central executive network
FAAH	Fatty acid aminohydrolase
FEP	First-episode psychosis

FKN	Fractalkine
fMRI	Functional magnetic resonance imaging
GABA	Gamma-aminobutyric acid
Glu	Glutamate
HAB	High-affinity binder
ICC	Intra-class correlation coefficient
IFNγ	Interferon gamma
IL	Interleukin
LAB	Low-affinity binder
LPS	Lipopolysaccharide
LTD	Long-term depression
LTP	Life-time prevalence
MAB	Medium-affinity binder
MAGL	Monoacylglycerol lipase
mGluR	Metabotropic glutamate receptor
MMP	Mitochondrial membrane potential
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
NAPE	N-acylphosphatidylethanolamine
NAPE-PLD	NAPE phospholipase D
NMDA	N-methyl-D-aspartate
OSEM	Ordered subset expectation maximization
PET	Positron emission tomography
PPAR	Peroxisome proliferator-activated receptor
PV+	Parvalbumin positive
SN	Salience network
SCID-I	Structured clinical interview for DSM-IV axis I disorders
SNP	Single nucleotide polymorphism
SUV	Standardized uptake value
mSUV	Modified standardized uptake value
T1w	T1-weighted
TGFβ	Transforming growth factor beta
THC	Δ^9-tetrahydrocannabinol
TNFα	Tumor necrosis factor alpha
TSPO	Translocator protein
VDAC	Voltage-dependent anion channel
V_T	Distribution volume

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I Laurikainen H, Tuominen L, Tikka M, Merisaari H, Armio RL, Sormunen E, Borgan F, Veronese M, Howes O, Haaparanta-Solin M, Solin O, Hietala J; METSY group. Sex difference in brain CB1 receptor availability in man. Neuroimage, 2019: 184:834–842.

II Borgan F*, Laurikainen H*, Veronese M, Marques TR, Haaparanta-Solin M, Solin O, Dahoun T, Rogdaki M, Salokangas RK, Karukivi M, Di Forti M, Turkheimer F, Hietala J, Howes O; METSY Group. In Vivo Availability of Cannabinoid 1 Receptor Levels in Patients With First-Episode Psychosis. JAMA Psychiatry, 2019; 76(10):1074–1084.

III Laurikainen H, Vuorela A, Toivonen A, Reinert-Hartwall L, Trontti K, Lindgren M, Keinänen J, Mäntylä T, Paju J, Ilonen T, Armio RL, Walta M, Tuisku J, Helin S, Marjamäki P, Hovatta I, Therman S, Vaarala O, Linnaranta O, Kieseppä T, Salokangas RKR, Honkanen J, Hietala J, Suvisaari J. Elevated serum chemokine CCL22 levels in first-episode psychosis: associations with symptoms, peripheral immune state and in vivo brain glial cell function. Translational Psychiatry, 2020; 10(1):94–107

*: Equal contribution

1 Introduction

There has been considerable progress made in elucidating the etiology of psychotic disorders, such as schizophrenia in the new era of neuroscience. Increasingly sophisticated imaging methods have been successfully employed to clarify our understanding of the biological mechanisms behind these disorders. The demonstration of the role of dysregulation of the dopaminergic system in schizophrenia and bipolar psychoses has served to highlight the potential of these novel research tools. We now seem to have gained at least a partial understanding of how positive symptoms are generated through disturbances of behavioural salience attribution.

The current golden standards of psychiatric disorder categorization, dictated by historical necessities, have however likely hampered efforts to fully understand these complex disorders with overlapping phenotypes, genetic associations, environmental associations, and biologies. Transdiagnostic research strategies have been increasingly applied to delineate possible diagnostic subtypes, and to understand the intertwining etiological mechanisms, intermediate phenotypes, and their associations with environmental factors.

Currently, the field of psychosis research seems to have progressed to studying the upstream pathophysiological mechanisms, such as the endocannabinoid and immune systems, which could contribute to the dopaminergic dysregulation. The task is daunting since the dopaminergic disturbance is most likely a result of heterogenous converging forces. These forces act through multiple complex biological systems, which exist in a non-linear interaction with each other and the environment. It is increasingly unlikely that there will be breakthrough discoveries indicating that the etiology of schizophrenia can be viewed as a discrete diagnostic entity since there are currently a huge number possible contributing factors and these are likely to increase in the future.

Modern treatments of psychotic disorders are unable to adequately address the impacts of negative symptoms, cognitive dysfunction, loss of occupational capacity and treatment resistance. Transdiagnostic research has however already demonstrated that understanding the biology behind symptom domains can translate into novel treatments. An appreciation of the fundamental pathophysiology can also

lead to the development of clinical tools for characterizing individual patterns of symptoms and recognition of posited diagnostic subtypes. If the individual needs of patients are to be met, we have to improve our understanding of psychosis beyond dopamine dysregulation.

2 Review of the Literature

2.1 The duality of psychotic disorders: discrete syndromes and transdiagnostic continuums

Psychosis is a disordered mental state in which internal representations of reality are incomprehensibly distorted or disconnected from the external reality shared by other individuals but neither the external nor internal realities can however be defined objectively. To address this constraint, behaviour and phenomenology are interpreted as expressions of internal realities, which are then projected against cultural backgrounds as an approximation of how internalizations of external reality are normatively expressed.

The phenomenology of psychotic states has been thoroughly described in the literature: realities can be distorted in regards to the salience of external events, thought content, sensory experiences and their interpretation, the syntax of thought, volition and motivation, and as an epiphenomenon of the previous, behaviour. (Jaspers, 1997; McGlashan et al., 2010) Typical syndromes of psychotic phenomena have been described throughout history. (Bleuler, 1950; *Diagnostic and statistical manual of mental disorders*, 1994; Kraepelin, 1919) Schizophrenia was classically considered to express fundamental symptoms, which correspond to modern descriptions of negative symptoms, while positive symptoms were seen as accessory due to their episodic nature. (Arantes-Goncalves et al., 2018) These historical concepts can be seen as manifestations of the influence of a core neurodevelopmental liability trait, and the many forms of psychotic states induced by stress.

Modern diagnostic practice acknowledges the existence of many psychotic syndromes such as schizophrenia, mania, psychotic depression, schizoaffective disorder, delusional disorder, brief psychotic disorders, substance induced psychotic disorders, psychotic disorders due to a general medical condition and psychotic disorder not otherwise specified. (*Diagnostic and Statistical Manual of Mental Disorders*, 2013) These syndromes of typically co-occurring symptoms have been treated as categorical medical disorders although their definitions are disconnected from both pathophysiology and etiology.

The severity and quality of symptoms of psychotic disorders can be evaluated and compared between patients, in clinical practice or in research settings, with the

use of structured interviews such as the brief psychiatric rating scale (BPRS). (Ventura et al., 1993) Although psychotic disorders present with considerable heterogeneity of symptom severity and quality, the symptoms can be further simplified into cross-culturally stable symptom classes of manic excitement/disorganization, anxiety/depression, negative symptoms and positive symptoms. (Ruggeri et al., 2005) Symptoms are deemed positive when the patient's sensorium or thoughts contain elements outside the normative field of experiences. Negative symptoms are consistently described as a diminution of normative mental functions for example, auditory hallucinations are positive while a loss of volition is a negative trait. In addition, psychotic disorders present with variable degrees of cognitive deficits, which can be evaluated with batteries of neuropsychological tests. (W. Li et al., 2020; Nuechterlein et al., 2008) The latent symptom domains of positive, negative or cognitive symptoms can be used to understand their underlying biological phenotypes.

Genetic studies have suggested that a large part of the genetic vulnerabilities of psychosis are not specific for one disorder as they overlap to affect the individual's liability to suffer from various psychiatric disorders, such as schizophrenia and bipolar disorder. (Anttila et al., 2018) It is also notable that subsyndromal positive symptoms of psychotic disorders are present in up to 8% of the general population worldwide. (Nuevo et al., 2012; Pignon et al., 2018; van Os et al., 2009) Furthermore, attenuated psychotic experiences are even more commonly present in the context of various other non-psychotic psychiatric disorders; they confer a liability for later episodes of schizophreniform psychosis. (Werbeloff, 2012) It can be claimed that at least some of the etiological factors, and their resulting biological phenotypes, form a continuum of psychosis liability across the general population regardless of whether the symptoms of psychosis emerge at the level of diagnosable disorders. (van Os et al., 2009; van Os & Reininghaus, 2016) The high population frequencies of many of the genetic associations with schizophrenia support this view. (Ripke et al., 2014) Negative and disorganization symptoms also seem to present as a liability-associated continuum in the general population. (Dominguez et al., 2010) However, the domains of negative and cognitive symptoms seem to predate positive symptoms, and they also differentiate the psychotic disorders from each other. (Sheffield et al., 2018; Strauss et al., 2016)

Rare copy number variation (CNV) risk genes are associated with relatively high odds ratios of developing schizophrenia, and they are also evident in non-psychotic developmental syndromes presenting with intellectual disability, **Figure 1**. (Singh et al., 2022) CNVs also seem to be an independent contributing factor to cognitive impairment in schizophrenia. (Thygesen et al., 2021) A neurodevelopmental diathesis due to multiple gene-environment interactions is suggested to predispose to the emergence of positive symptoms in response to stress in adolescence or early

adulthood. (van Os et al., 2009) The liability for psychosis seems to be a transdiagnostic population continuum with degrees of disorder specificity according to the relative abundance of negative and cognitive symptoms.

Nonetheless, psychiatric syndromes are defined as discrete entities according to certain subjective phenomena. According to current diagnostic conventions, the presence of psychotic symptoms can lead to a variety of diagnoses ranging from brief psychotic disorders to schizophrenia. (First et al., 2002) The established diagnostic manuals are designed to optimize the reproducibility of psychiatric diagnoses independent of the clinical setting, which is achieved by disorder definitions based on the typical syndromes of symptoms.

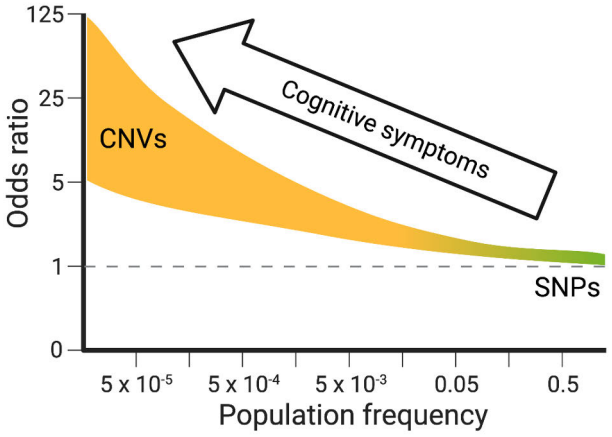


Figure 1. The relation of the odds ratio of developing schizophrenia associated with a given genetic risk factor, and the population frequency of the said risk factor. (Singh et al., 2022) The probability of cognitive impairment rises when high odds-ratio CNVs are present. (Thygesen et al., 2021) SNP (green): single nucleotide polymorphism; CNV (yellow): copy number variation.

Since causal associations between symptoms and underlying biological factors, such as genetic factors, cannot be ascertained by phenomenology alone, efforts have been made to link the gap between phenomenology and genetics of psychotic disorders via the study of intermediate phenotypes and research domain diagnostic criteria. Multimodal taxometric analyses from these research efforts suggest that the biological underpinnings of psychotic disorders span across diagnostic borders, but also cluster into several underlying biotypes. (Clementz et al., 2022; Clementz et al., 2016) For example, cognitive symptoms are differentially present in transdiagnostic biotypes of psychotic disorders. (Clementz et al., 2022)

The separation of psychotic disorders into discrete entities based on symptom syndromes does not seem to be an optimal strategy for studying the biological etiologies underpinning psychotic disorders. Due to the same issues, current diagnostic practices might also lead to under- or over-estimation of the potential clinical impact of biological treatments, since specific pharmacodynamic effects are targeted at heterogenous psychopathological mechanisms. Simplifying the structure of psychotic phenomena is thus necessary for diagnostic practice and research. However, descriptions of the individual's symptoms remain important in the

treatment process. Understanding the cultural, social and behavioural effects of psychotic interpretations facilitates rehabilitation and risk management efforts regardless of the degree of symptomatic remission achieved through pharmacological treatment.

Examples of descriptions of selected presenting symptoms, as categorized by the structured interview for prodromal syndromes, are presented in **Table 1**.

Table 1. Examples of common positive symptoms of psychotic disorders.

Symptom Class	Description	Phenomenology	Implication
Delusion	Bizarre and paranoid explanations of thought censorship via cosmic radiation emanating from ill-meaning ancient entities	"It feels as though. I mean, they don't want me to have any positive thoughts. They have total control over my mind and are trying to erase my memories. I think they are trying to take me as their puppet."	Social dysfunction and withdrawal, affective symptoms, cognitive interference, occupational functioning.
Hallucination	Auditory hallucinations of several persons malevolently commenting on physical attributes	"The neighbors have really ramped it up. I could not sleep at all last night. They were yelling such mean things that I was afraid they would break into my apartment to hurt me. They were yelling at my ugly nose, how stupidly infantile my bed sheets are. Even how I looked while sitting at the toilet. I was so embarrassed. Can't you call their landlord and ask them to make them stop?"	Insomnia, cognitive interference, affective symptoms.
Disorganization	Derailment of thought during speech	"Thanks for asking. I have been in a much better mood recently. I bought this nice sweater from the flea market. Those merchants are always trying to sell you something you don't need. I really should go buy new curtains, but there is no time. No time like now to make a change, isn't it so?"	Social dysfunction, inability to communicate needs, occupational functioning.

2.2 Epidemiology of psychotic disorders

Psychotic disorders will afflict approximately 2.8–4.5% of the population in their lifetime. (Bogren et al., 2009; Jacobi et al., 2004; Perälä et al., 2007) In the Finnish "Terveys 2000" -study, the most prevalent singular psychotic disorder was schizophrenia with a lifetime prevalence (LTP) of 0.87%, followed by a 0.45% LTP

of psychotic disorder not otherwise specified, and 0.35% LTP of schizoaffective disorder. (Perälä et al., 2007) The LTP values of the affective psychosis disorders, bipolar I disorder and major depressive disorder with psychotic features, were 0.24% and 0.35% respectively. (Perälä et al., 2007) In addition to causing subjective distress and a reduction in the quality of life, (Neil et al., 2018) psychotic disorders pose a significant financial burden in the developed countries. (Andlin-Sobocki & Rossler, 2005)

2.3 Gender differences in psychotic disorders

Males have a higher prevalence of schizophrenia than females (1.4:1), (Hwang et al., 2020) and the age of onset of psychotic disorders is known to differ between the genders. (Giordano et al., 2021; Kraepelin, 1919) The typical age of onset of schizophrenia in males ranges from the late teens to the early twenties, while in females, the age of onset is bimodal. The first peak of onset occurs in the late twenties and a second smaller peak occurs after 40 years of age. (Giordano et al., 2021) Possible explanations for these differences include differences in genetics, neurodevelopment, endocrine functioning, but also they may involve psychological and social factors.

The later onset peak in females has been suggested to relate to diminishing peri- and post-menopausal 17-β-estradiol levels, a hormone postulated to be protective against psychotic episodes. (Giordano et al., 2021; Hwang et al., 2020) Estrogen has also been associated with the emergence of psychosis during the first incidence peak as demonstrated by the increased incidences of psychosis in states with decreased circulating estrogen levels, low estrogen phases of the ovarian cycle and other hypoestrogenic states. (Hwang et al., 2020; Riecher-Rössler, 2016) Hypothalamo-pituitary-gonadal dysfunction, presenting as low levels of estrogen and increased prolactin, is commonly seen in acute psychotic states, and it has been reported to occur prior to initiating antipsychotic medication. (Riecher-Rössler, 2016) Notably, it is believed that estrogen modulates the dopaminergic system at various levels of the brain. (Hwang et al., 2020) Differences in sex hormone levels between genders, and within the reproductive cycles and events of females, could correspondingly mediate the predisposition to the positive symptoms of schizophrenia by modulating mesolimbic dopaminergic function. (Hwang et al., 2020)

Clinical presentation and the course of illness also differ by gender. Males suffering from schizophrenia typically have worse premorbid levels of functioning, greater degrees of negative symptoms, and less severe affective symptoms compared to females with schizophrenia. (Giordano et al., 2021) Similar gender differences exist in persons at a clinical high risk for psychosis. (Menghini-Müller et al., 2019)

There seems to be no significant gender differences in either the prevalence or incidence of bipolar disorder, (Diflorio & Jones, 2010) or psychotic depression, even though females are more prone to experience depression. (Bogren et al., 2018) However, females seem to be more prone to hypomanic and mixed states when compared to males, and the risk for symptom relapse of bipolar disorder is greatly increased during the first month post-partum. (Diflorio & Jones, 2010) Males present more often with hallucinations in psychotic depression, and with hallucinations and Schneiderian first-rank-symptoms in the context of bipolar mania compared to females. (Diflorio & Jones, 2010; Park et al., 2015)

2.4 Treatment of psychotic disorders

Psychotic disorders are treated based on individual treatment plans, which consider the symptom profile, phase of illness, social circumstances, functional and cognitive capacity, somatic health and cultural background of the individual suffering from the disorder. ("Kaksisuuntainen mielialahäiriö. Käypä hoito -suositus," 2021; "Skitsofrenia. Käypä hoito -suositus," 2022)

Psychosocial treatments and treatment paradigms, such as family interventions, social cognition and interaction training, cognitive remediation therapy, cognitive behavioural therapy, assertive community treatment, early intervention services, meta-cognitive training, psychoeducation, befriending, and adherence therapy, have been found to be variably effective for treatment and rehabilitation of psychotic disorders. (Bighelli et al., 2021; Y. Li et al., 2020; Solmi et al., 2023) These treatments are underused although various clinical guidelines do recommend their use. This is probably due to the poor availability of expertise and lack of resources. (Bighelli et al., 2016)

Antipsychotic medication remains the mainstay of psychosis treatment. ("Kaksisuuntainen mielialahäiriö. Käypä hoito -suositus," 2021; "Skitsofrenia. Käypä hoito -suositus," 2022) The real-world effectiveness of antipsychotic medication has been best demonstrated by observations of re-hospitalisation hazard ratios of 1.63-7.28 after the patient discontinues his/her antipsychotic medication, when compared to continuing medication. (Tiihonen et al., 2018) Clozapine and long-acting injectable antipsychotics provide the best protection against symptom episodes requiring re-hospitalisation. (Tiihonen et al., 2017) The use of antipsychotics in clinical studies with selected study populations does not increase short-term mortality, (Schneider-Thoma et al., 2018) and results from nationwide register studies indicate that antipsychotics may be able to reduce long-term mortality by as much as 60%. (Taipale et al., 2018; Tiihonen et al., 2018) The population level effectiveness of antipsychotics is however hampered by the large rates of treatment discontinuation after symptom episodes requiring hospitalization.

(Rubio et al., 2021) In addition, a sizable proportion of patients are intolerant of antipsychotic treatment due to the development of extrapyramidal and other side effects; about 30% of individuals remain resistant to treatment with non-clozapine antipsychotic medications, and clozapine does not provide a treatment response in 1 out of 3 treatment-resistant individuals. (Sinclair & Adams, 2014) After clozapine treatment failure, the clinician has little in the way of providing treatment rationally due to the paucity of evidence of efficacious alternatives to clozapine treatment. (Sinclair & Adams, 2014) Another problem lies in the domain of negative symptoms. Statistically significant, but clinically insignificant treatment effects have been evident in trials comparing antipsychotic medications, glutamatergic medications, antidepressants, brain stimulation, psychological interventions and combination treatment regimens to treatment as usual. (Fusar-Poli et al., 2015) The lack of effective treatments for negative symptoms is especially troublesome for patients and their families, since negative symptoms mediate real world functioning more robustly than positive symptoms. (Ventura et al., 2009)

2.5 Prognosis of psychotic disorders

The functional prognosis of psychotic disorders remains generally poor despite decades of vigorous clinical research efforts, drug development, trials of psychosocial rehabilitation and advances in neuromodulative treatments. (Peritogiannis et al., 2020) In a worldwide multinational study of clinical and functional outcomes of 11078 persons suffering from schizophrenia, 66.1% achieved clinical remission and 25.4% achieved functional remission during a 3-year follow-up. (Haro et al., 2011) It has been reported that the median proportion of persons with schizophrenia or schizoaffective disorder who meet the remission criteria for clinical and social/vocational functioning for at least two years is about 12–13%. (Jaaskelainen et al., 2013; Mustonen et al., 2018) In other words, only one out of seven individuals with schizophrenia achieves a long-term recovery. Compared to schizophrenia, other psychotic disorders, such as bipolar disorder, psychotic depression and brief psychotic disorders have a better outcome, but still worse when compared to non-psychotic depression. (Fusar-Poli et al., 2016; Nietola et al., 2018) The duration of untreated psychosis, premorbid functioning, severity of negative symptoms, and genetic liability to schizophrenia all associate with a worse long-term functional outcome. (Peritogiannis et al., 2020) In addition, better psychosocial functioning and fewer negative symptoms predict a diagnosis of a bipolar disorder after the first episode of psychosis. (Salagre et al., 2020) There seem to be no gender differences in the outcome of schizophrenia although males with schizophrenia have more severe negative symptoms and worse premorbid functioning than females. (Giordano et al., 2021)

It is probable that only a fraction of psychosis risk factors have been currently identified. Some risk factors are relevant only when they occur during specific neurodevelopmental phases, while others are more proximally involved in the emergence of frank psychosis. Prognoses can be roughly compared between disorders; for example schizophrenia has an overall worse prognosis compared to brief psychotic disorders. However, given the diversity of risk factors, their temporal sequences and mutual interactions, the prognosis of psychosis is bound to be heterogenous even within specific disorders.

2.6 Risk factors and manifestations of psychotic disorders

Multiple lines of research have detected associations between the psychotic disorders and genetics, (Ripke et al., 2014; Tandon et al., 2008) psychosocial factors, (Tandon et al., 2008) cannabis use, (Mustonen et al., 2018) immunology, (Goldsmith et al., 2016) metabolism, (Henderson et al., 2015) and neurobiological factors (Keshavan et al., 2008). Cumulating evidence thus suggests there is a multifactorial etiology behind these disorders, such as schizophrenia, which in global terms is the most prevalent psychotic mental illness. (Jablensky et al., 1992) Psychosis can be viewed as a manifestation of genetic, biological and environmental liabilities, which present in differential contexts of homeostatic stress depending on the individual constellations of these liabilities. For example, some individuals might become psychotic only in the context of severe major depression, while others express symptoms of schizophrenia as a result of more subtle homeostatic stress.

2.6.1 The genetics of psychotic disorders

The genetic liability to psychotic disorders in a population varies considerably depending on the disorder. Twin-studies have provided us with estimates of the degree of variability in the population prevalence of psychotic disorders that is due to genetic variation in individuals, or more aptly, the heritabilities of psychotic disorders. Heritabilities range from 39% in psychotic depression to values as high as 81% in schizophrenia. (Lyons et al., 1998; Sullivan et al., 2003) Estimates from studies utilizing national registries have suggested more modest but still notable heritabilities of 67% for schizophrenia and 62% for bipolar disorder. (Wray & Gottesman, 2012) Although genetic variability seems to play a major role in the etiology of psychotic disorders, the specific genes behind the liabilities have remained strangely elusive. This has probably been due to a combination of the polygenic nature of schizophrenia, and the very modest odds ratios of developing schizophrenia attributable to common risk genes. Recent large-scale studies of

hundreds of thousands of participants have fortunately provided us with more insights into the common single-nucleotide polymorphisms (SNP) associated with schizophrenia. The heritability estimates of schizophrenia attributable to the recognized SNPs have ranged from 21% to 31%, depending on the sample. (Trubetskoy et al., 2022) The population frequency of liability associated SNPs is in fact so common, that their singular effect in increasing the odds for developing schizophrenia is comparable to many well-established environmental risk factors, such as an urban upbringing. (Trubetskoy et al., 2022) On the other hand, rare recurrent copy number variants (CNV) are associated with a significantly larger individual risk, but their scarcity indicates that they have only a minute role in explaining the heritability of schizophrenia at the population level. (Singh et al., 2022)

The functional implications of the common SNPs and rare CNVs are, however, important for understanding the pathophysiology of psychotic disorders. The genetic backbone of psychiatric disorders seems to include both genes that are specific to certain disorders, and genes that increase the risk for multiple disorders. (Anttila et al., 2018) The shared genotypes appear to be located in evolutionally conserved areas of the genome, which suggests that variations in these loci can result in a transdiagnostic liability through dysfunctions of highly conserved biological functions. (Romero et al., 2022) The overlap of risk alleles is considerable for severe mental disorders such as schizophrenia and bipolar disorder. (Anttila et al., 2018; Romero et al., 2022) This implies that certain aspects of these disorders, for example a liability to suffer from a psychosis, is also transdiagnostic to some degree, and that the phenotypic variance between the disorders is explained by disorder specific alleles or environmental factors.

Genome-wide association studies of specific disorders have provided us with a more detailed understanding of the functional relevance of genotypic variance. The polygenic load for schizophrenia is concentrated on functions which are not only relevant for singular neuron types, but also for the functioning of a wide variety of neurons: pre- and post-synaptic cellular events, regulation of intracellular calcium levels, metabotropic glutamate receptors, N-methyl-D-aspartate (NMDA) receptor subunits, synaptic adhesion molecules and various other regulators of synaptic functions. (Trubetskoy et al., 2022) These observations suggest that the synapse lies at the root of the problem, and that the extensively characterized bio- and phenotypes of psychotic disorders are secondary to these genetic faults. (Howes & Onwordi, 2023)

2.6.2 Environmental risk factors

Stressful life events, stress from migration into a dissimilar culture and social adversities confer a heightened risk for psychosis. (Keshavan et al., 2008) Childhood

adverse and traumatic experiences predispose the individual to a wide range of psychiatric disorders. (Salokangas, 2020) In his thesis, Salokangas (2020) observed that the type of adverse childhood experience has relevance with regards to the predisposition to different disorders. For example, emotional abuse tended to be associated with depression, anxiety and substance abuse, while physical abuse was more specifically linked to psychotic and bipolar disorders. The interaction of genes and environment can be seen as more proximal to neurodevelopment in other recognized environmental risk factors: obstetric and perinatal complications, severe adversity suffered by the mother during the first trimester, nutritional deficiency, cannabis use and severe infections. (Tandon et al., 2008) Finally, risk factors such as urbanicity, older paternal age as well as birth during winter demonstrate the complex interactions between genes, neurobiology and the social environment. (Tandon et al., 2008)

2.6.3 Structural brain changes

The most stable neurobiological facets of brain morphological changes in schizophrenia are those of reduced total brain volume and enlarged later ventricle volumes. (Keshavan et al., 2008) The histological ex vivo evidence of the pathology behind these changes has revealed robust reductions in neuropil, a synaptically dense tissue compartment comprised of glial cells, dendrites and axons. (Keshavan et al., 2008) It could be speculated that in vivo analyses using structural magnetic resonance imaging (MRI) could provide deeper insights into the morphology of regions of interest. Large scale efforts to characterize the fine details of the structural pathology of psychotic disorders, given their heterogenous nature, have recently been published as mega-analyses involving thousands of patients who have been studied using MRI methods to measure cortical and subcortical structures. The largest reductions of MRI volume in individuals with schizophrenia as compared to healthy controls are seen in total brain volume, total grey matter volume, and volumes of the hippocampus, amygdala, thalamus and nucleus accumbens. (Haijma et al., 2013; Van Erp et al., 2016) Reductions of medial frontal cortex thickness are seen already in individuals with a clinical high-risk for psychosis and first episode psychosis, while chronic schizophrenia additionally is associated with comparatively larger reductions in the thickness of insular and temporal cortical regions. (Y. Zhao et al., 2022) The effects of the severity and duration of illness can also be seen in individuals with medicated schizophrenia as larger volume reductions of total grey matter as well as increased ventricle size when compared to medication naïve schizophrenia. (Haijma et al., 2013) A comparison of volume reductions in cortical and subcortical grey matter between schizophrenia, bipolar disorder and major depressive disorder reveals relatively smaller cortical volumes and thicknesses in the

frontal cortex, temporal cortex, hippocampus and thalamus of individuals suffering from schizophrenia and bipolar disorder in comparison to those suffering from major depressive disorder. (Cheon et al., 2022) Similar measurements from individuals with schizotypy, a proposed milder clinical presentation of vulnerability to schizophrenia, have revealed a different pattern of larger cortical volumes and thicknesses in the orbital frontal and prefrontal cortices as compared to controls. (Kirschner et al., 2022) This suggests that at least some of the brain changes seen in the context of psychotic disorders are due to state rather than trait. It is however impossible to distinguish the effects of state from trait using cross-sectional study designs. Additionally, some of the findings, for example from individuals with schizotypy, on the other hand might reflect beneficial compensatory changes associated with resilience to psychosis.

White matter pathology is present, and partly overlapping, in schizophrenia and bipolar disorder. (G. Zhao et al., 2022) When compared to healthy controls, both diagnosis groups show diminished fractional anisotropy, a measure reflecting the structural integrity of white matter, and reduced white matter volume in the corpus callosum and anterior corona radiata. Furthermore, differential decreases in fractional anisotropy and white matter volume are also present in the left cingulum and internal capsule. (G. Zhao et al., 2022)

2.6.4 Functional brain changes

The evaluation of brain glucose metabolism using fluorodeoxyglucose positron emission tomography (PET) further supports the concept of frontal pathology in schizophrenia. Individuals with schizophrenia present with decreased frontal metabolism, both absolute and relative to whole brain metabolism, when compared to healthy individuals. (Townsend et al., 2022) Consistently, patients with schizophrenia spectrum disorders also express patterns of frontal cortical hypoperfusion, and hyperperfusion of the putamen, which are associated with negative symptoms and positive symptoms respectively. (Percie du Sert et al., 2023)

Zahid et al. (2023) systematically reviewed the studies examining the associations between functional MRI (fMRI) blood oxygen level dependent (BOLD) signal and magnetic resonance spectroscopy (MRS) measures of glutamate (Glu) and gamma-aminobutyric acid (GABA) in psychosis. The BOLD signal represents the magnitude of oxygen consumption (deoxygenated blood) in a given measurement volume. The BOLD signal is thus thought to reflect the amount of neural activity, since under normal conditions, neurons use oxygen for cellular respiration in order to obtain the energy needed for cellular functions. Overall, the evidence suggests that the normative positive associations of Glu and anterior cingulate cortex BOLD signal are stronger in psychosis patients during cognitive tasks, and weaker during rest

when compared to healthy controls. Zahid et al. (2023) also reported that the inverse association between anterior cingulate cortex GABA and BOLD signal in healthy controls was weaker in psychosis patients. These concepts are evidence of an imbalance between excitatory Glu and inhibitory GABA cortical activity in psychotic disorders.

The connectivity of the brain can be measured in terms of structural connectivity with diffusion weighted MRI, and functional connectivity using fMRI. Functional connectivity is theoretically closely related to structural connectivity, although structural connectivity is strictly speaking not a functional measure. In most publications, structural connectivity has been consistently and globally reduced in chronic schizophrenia, while a significant number of studies report unchanged functional connectivity in first episode psychosis compared to controls. (Fornito et al., 2012; Pettersson-Yeo et al., 2011) Functional connectivity seems to be more complexly affected in psychosis, since some brain areas show reduced connectivity, while some displayed increased connectivity despite the co-localized reduced structural connectivity as compared to healthy controls. (Fornito et al., 2012; Pettersson-Yeo et al., 2011) It should be noted that measurements of functional connectivity are more prone to methodological bias, such as from the non-selective nature of global BOLD signal corrections, which might explain some of the heterogeneity. (Fornito & Bullmore, 2015)

Brain activation patterns during specific mental tasks can also be measured with fMRI in task-activation studies. According to the review conducted by Mwansisya et al. (2017), these studies show an altered capacity for recruiting activity in the prefrontal and temporal areas during cognitive tasks. However, due to methodological uncertainties in fMRI paradigms, and since heterogenous tasks have been used in a wide variety of studies, it is not straightforward to conduct an overall interpretation of the results.

Examining the functional connectivity of the whole brain reveals canonical large-scale networks consisting of temporally correlated neural activity. For example, typical temporospatial patterns of brain activity become more pronounced when an individual is not engaged in interacting with the outside world, and different patterns of activity arise when he/she is engaged in detecting and focusing on outside stimuli. The resulting patterns of associated BOLD signals are correspondingly the Default mode network (DMN) and the Salience network (SN). In addition, complex problem-solving and sustained attention engages the frontoparietal areas associated to the Central executive network (CEN). It has been speculated that the network dysfunction in schizophrenia is due to an inability to shift the balance of network activity between the opposing DMN and CEN, because an aberrantly activated SN is unable to mediate the deactivation of the DMN and trigger the engagement of the CEN. (Nekovarova et al., 2014) This has been suggested to be a secondary

phenomenon to the dopaminergic dysregulation in the striatum. (Menon et al., 2022; Rossler et al., 2020)

These disturbances in hypometabolism, excitation-inhibition balance, structural connectivity, large-scale functional network imbalance, and the capacity to engage task-related activation can be seen as neurodevelopmental traits, secondary dynamic states, or a combination of both.

2.6.5 Summary

The impact of the genetics of schizophrenia stems from the neurodevelopmental effects of inheriting an unfortunate constellation of common genetic variants, and more infrequently, catastrophic copy number variations. These neurodevelopmental effects influence the structure of neurons, as well as immune and synaptic functions. A multitude of genetic and environmental factors modulate synaptic functioning during neurodevelopment. The individual combinations of these factors converge to translate a genetic liability for psychosis into widespread structural and functional brain changes with trait-like as well as context-dependent features. Altogether, the neurobiological changes associated with psychotic disorders can be conceptualized as network problems with an emphasis on frontal and temporal cortex dysfunction.

2.7 Neurobiological and immunological mechanisms in psychotic disorders

2.7.1 Brain network hypotheses of psychotic disorders

2.7.1.1 An introduction to brain network dysfunction

It is necessary to mention first, that the functioning of the human nervous system is critically dependent on two classes of neurons: the excitatory glutamatergic projection neurons, and the inhibitory GABA interneurons. The dopaminergic neurons, along with the other modulatory neurotransmitter systems arising from the midbrain, tegmentum and brainstem, act to modulate the processing of information within cortico-cortical projections and cortico-subcortical-interconnections. Glu and GABA are the primary means of transmitting action potentials from the pre- to postsynaptic neurons of these cortical projections and connections.

Information can be coded within these neural networks via the magnitude of the hyperpolarization, molecular mechanisms, or spatial and temporal characteristics of neural signals. Various filtering mechanisms are embedded within the laminar and columnar organizations of the cortex, for example to laterally inhibit neural activity

adjacent to a specific signal in order to increase the magnitude of the salient signal compared to adjacent unrelated neural activity, or in other words to increase the signal-to-noise ratio.

The modulatory neurotransmitter systems act to integrate homeostatic signals and the global state of the brain by scaling the balance of excitation and inhibition, or by fine-tuning filtering mechanisms to suit the perceived environmental situation. Dysfunctional modulation can lead to dysfunctional cognitive and behavioural responses, but on the other hand, a gross dysfunction of the glutamate or GABAergic neurons leads to severe states within the networks. For example, unopposed excitatory stimulation evokes an epileptic state. Dysfunctions of smaller magnitude can cause subtler errors in the temporospatial accuracy of excitation and inhibition, a disruption of connectivity patterns, and a propensity towards a decreased signal-to-noise ratio. These altered brain states can lead to problems in cognitive processing and a disruption of the connection between neural representations of the environment and the environment itself, for example as seen in the sensory distortions triggered by Δ^9-tetrahydrocannabinol (THC) intoxication. Finally, a multitude of minute flaws and liabilities can be embedded into synapses during neurodevelopment. These flaws can predispose to either the disorganized or disordered information processing described above – especially during homeostatic stress. More details of the matters discussed in this chapter can be found in Kandel (2021) and references therein.

2.7.1.2 The dopamine hypothesis of schizophrenia

Although antidopaminergic medications have been available since the late 1950s, the first direct in vivo evidence of central dopaminergic dysfunction in schizophrenia emerged as late as in the era of second-generation antipsychotic medications. (Hietala et al., 1995) These early findings have since been consolidated and elaborated into one of the main theoretical models of how psychotic symptoms emerge, and why antidopaminergic medications are effective in their treatment, (Howes et al., 2012; Howes & Kapur, 2009; Howes & Nour, 2016), **Figure 2**. The overlapping genetics of severe psychotic states seem to be consistent with this theory, since both patients suffering from bipolar states and schizophrenia, (Jauhar et al., 2017) and delusional disorder, schizophrenia and other psychotic disorders, (Cheng et al., 2020) have a similarly elevated presynaptic dopamine synthesis capacity when compared to healthy individuals. However, due to the polyfactorial nature of psychotic disorders, and how they are categorized based on phenomena secondary to biology, it is not unexpected that there is some degree of pathophysiological variation.

Early singular observations of diminished presynaptic dopamine synthesis capacity in treatment-resistant schizophrenia have been subsequently repeated at the

group level, which reinforces the concept that the etiology of psychotic disorders is heterogenous in nature. (Demjaha et al., 2012; Hietala et al., 1995) A similar heterogeneity between treatment responsive and treatment resistant patients has been documented using measures of resting state functional MRI connectivity and dopamine synthesis capacity in the striatal subregions. (Robert A. McCutcheon et al., 2020) It has been argued that dopaminergic dysfunction is the final result of a causal network of upstream pathology. (Howes & Nour, 2016) Differential distributions of primary pathological mechanisms between individuals would be consistent with the polyfactorial nature of psychosis and differences in pathophysiology would also explain why individual expressions of symptom domains are heterogenous.

2.7.1.3 Beyond dopamine: glutamatergic and neurodevelopmental hypotheses

The presence of a dysregulation of the glutamatergic system has been proposed to interact with the dopaminergic system to induce the increased striatal dopaminergic synthesis capacity seen in psychoses. The glutamate hypothesis of schizophrenia states that genetically dysfunctional NMDA receptors evoke an understimulation of inhibitory GABA interneurons, which in turn disinhibit corticostriatal pyramidal neurons and consequently overstimulate mesostriatal dopaminergic signalling. Ketamine, a NMDA-receptor antagonist, is known to provoke symptoms of psychosis dose-dependently, and also to increase mesostriatal dopamine release. Conclusive evidence to ascertain the glutamate hypothesis is however lacking due to the complexity of interactions of the ubiquitous excitatory/inhibitory control via glutamatergic and GABAergic neurons, and the lack of adequate measures to assess these systems at finer detail. Further, dopamine-type 2 receptor antagonists are able to alleviate only a part of the symptoms aggravated by administering ketamine, which suggests that there are complementary mechanisms unrelated to dopamine. For more details on this concept see Robert A McCutcheon et al. (2020) and references therein.

Another recently proposed overlapping hypothesis also posits that the dysfunction of cortical projections overstimulates mesostriatal dopamine neurons, but with the nuance that the dysfunction of pyramidal neurons, or their regulation, is at least partly of neurodevelopmental origin. (Howes & Shatalina, 2022) It is widely acknowledged that a progressive loss of grey matter volume occurs during the course of schizophrenia. (Vita et al., 2012) The volume changes seen in schizophrenia resemble those of normal brain aging, but it is as if they were happening earlier than would otherwise be expected. The phenomenon has thus been branded as a form of accelerated brain aging. Certain forms of accelerated brain aging have been linked

with increased levels of TNFα, (Klaus et al., 2022) and cognitive impairment, (Haas et al., 2022) but not to other clinical characteristics such as age at onset of psychosis, symptom severity, or use of antipsychotic medications. (Constantinides et al., 2023) Signs of accelerated brain aging can be seen in individuals with a bipolar disorder, first episode psychosis and major depression, with the effect sizes of accelerated age being highest in schizophrenia and lowest in major depression. (Blake et al., 2023)

It is not sure what proportion of accelerated brain aging is developmental in origin, and how much of it is explained by disease effects. The lower degrees of cortical gyrification in schizophrenia patients as compared to healthy controls suggests that the abnormal trajectories of cortical development begin during early life. (Zakharova et al., 2021) For example, the lower premorbid intelligence quotients of schizophrenia patients compared to healthy individuals also support the neurodevelopmental hypotheses. (Khandaker et al., 2011) Excess developmental pruning of inhibitory synapses is one possible explanation behind the proposed altered glutamatergic signaling and morphological changes. (Glausier & Lewis, 2013) In vivo evidence of decreased synaptic density is however currently restricted to individuals with chronic schizophrenia, (Onwordi et al., 2020; Radhakrishnan et al., 2021), and the precise temporal pattern and causes of this decrease are still unclear. Nonetheless, the multitude of genetic associations converging on synaptic structures and function suggests that the synapse, as a generic functional unit, is rendered liable to the effects of homeostatic stress, and that watershed populations of synapses are excessively eliminated during development. (Howes & Onwordi, 2023)

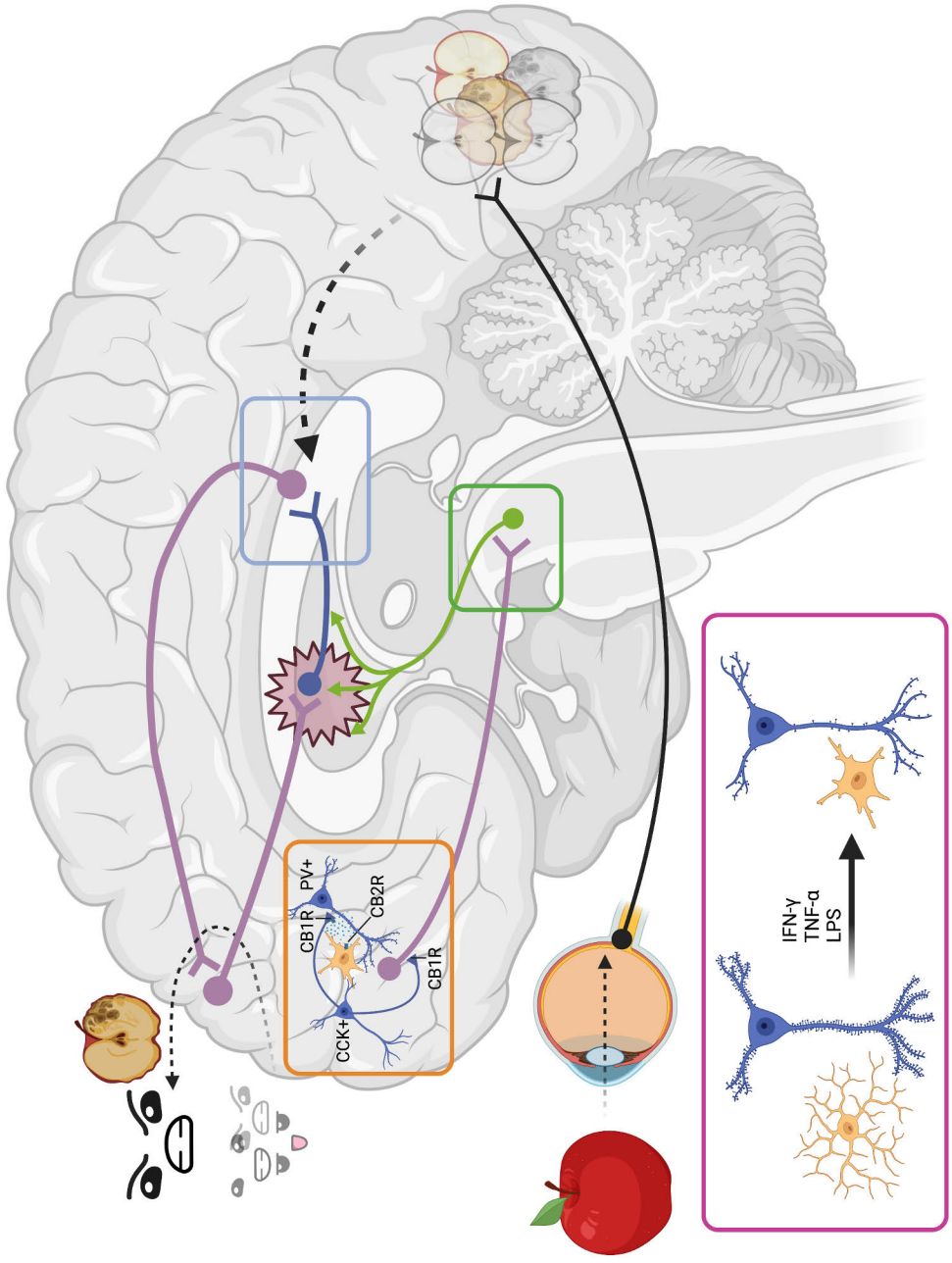
2.7.1.4 Excitation-inhibition balance

Neuroplastic changes occurring during development and continuing throughout adulthood govern the balance of excitatory and inhibitory activity in the cortex. A restriction to a narrow frame of excitatory activity is necessary for efficient information processing as are stable cortical states. For example, the periodic inhibition of pyramidal cells by PV+ GABA interneurons gives rise to gamma oscillations which can be recorded by electroencephalography (EEG). (Lisman, 2012) These oscillations provide a way to temporally segregate and code neural signals, and thus they can represent the higher level of processing needed to synchronize functional network activity. (Lisman & Buzsaki, 2008) For example, the abnormal gamma rhythms in the EEG have been associated with both cognitive impairments, (Mably & Colgin, 2018) and schizophrenia. (Sun et al., 2011) The balance of excitation and inhibition has been proposed to be dysregulated at the level of area-specific cortical microcircuits, oscillatory synchronization of circuits, or large-scale functional networks. (Lisman, 2012; Liu et al., 2021) Both GABA and

Glu neurons are involved in the regulation of the excitation-inhibition (E-I) balance during development. (Perica et al., 2022) Mechanism acting on GABA and Glu neurons are thus capable of dysregulating the intricate E-I balance. However, although the E-I balance is useful as a conceptual construct, it might not be optimal as a research endpoint. Theoretically associated sub-phenomena, such as network oscillations and Glu/GABA levels are quantifiable by EEG and MRS, but it is not entirely clear how the E-I balance can be measured directly.

2.7.1.5 Converging remarks

Two biological systems of interest, the endocannabinoids system (ECS) and the glia, are centrally involved in the regulation of neurodevelopment and plasticity. The ECS is also responsible for activity-dependent inhibition of cortical activity, (Lu & Mackie, 2016) while glial cells are involved in eliminating unnecessary synapses and extracellular debris, integrating information in neuronal networks, supporting neuronal metabolism, and maintaining an optimal extracellular environment for synaptic function. (Eraso-Pichot et al., 2023; Marinelli et al., 2023) The glial cells and the ECS are interconnected, and both are potentially able to contribute to disinhibiting the excitatory cortical projections to the midbrain, which are postulated to increase dopaminergic synthesis capacity in mesostriatal projections, and consequently, symptoms of psychosis. (Durieux et al., 2022; Tanabe & Yamashita, 2018) These systems will be discussed in more detail below. Furthermore, **Figure 2** is an illustration describing how the mesostriatal hyperdopaminergic state is suggested to relate to psychotic symptoms, cortical excitatory and neurodevelopmental dysregulation, as well as endocannabinoid regulation of cortical inhibitory interneurons and microglia.


PV+
CB1R
CB2R
CB1R
CCK+
IFN-γ
TNF-α
LPS

▲ **Figure 2.** An integrative view of the dopamine, neurodevelopmental and glutamate hypotheses of schizophrenia, and of how the endocannabinoid and immune systems might be related to these hypotheses. According to the dopamine hypothesis of schizophrenia, (Howes & Kapur, 2009) aberrant salience is attributed to thoughts and sensory experiences via mesostriatal dopaminergic projections (green pointed arrow) within the striatal parts of the cortico-striato-thalamo-cortical loop (fuschia star). Due to the dysregulated dopaminergic state of these projections, attributed salience is incoherent with current experiences and internal representations of external events. The precognitive experience of salience cannot be ignored, and consequently an explanation, which is detached from external reality, is formed. Blocking the mesostriatal dopaminergic tract with dopamine type 2 receptor antagonists alleviates the experiences of salience, and as time goes by the individual might be able to readjust his/her beliefs because the hyperdopaminergic state has stopped constricting interpretations of contradicting evidence. In this simplified example, the individual gazes upon a red apple. The image of the apple is conveyed through visual neural pathways (retina → lateral geniculate nucleus → primary visual cortex → association areas) to be finally interpreted within the frontal and limbic cortices with regards to valence (emojis) and the adequate behavioural response. The interpretation of the quality and valence of the experience is continually fine-tuned via inputs to the cortico-striato-thalamo-cortical loop (purple neuron as excitatory glutamate projections; blue neuron as inhibitory GABA projections). This loop integrates the homeostatic state, sensory context (blue box; dash arrow from the primary visual cortex), behavioural salience (green pointed arrow), and the state of other cortical areas to the experience. Cortical dysconnectivity and local neuronal dysfunction due to neurodevelopmental and/or environmental factors can lead to increasingly indiscrete boundaries of neural representations, as depicted here by superimposed images of various emotional states or apples. These indiscrete boundaries of representations predispose the individual to distorted sensory experiences (the path from the good apple on the bottom left to the bad apple on the upper left), loose associations, and incongruous emotional interpretations (emojis and dashed arrow on the left). When the distorted and incongruous representations are attributed with aberrant salience, the resulting phenomenology of firmly held bizarre and distorted thoughts/sensorium emerges. We must consider the cellular level (orange box) to integrate the other etiological theories, and the ECS and immune systems, to the dopamine theory of schizophrenia. A dysfunctional regulation of the excitatory projections from the cortex to the midbrain dopaminergic cells can be due to neurodevelopmental and/or disease effects. A disinhibition of these projections is proposed to be a result of inadequate genesis or excessive elimination of synapses on inhibitory cells. For example (orange box), the cortical projection neuron is normally inhibited via the parvalbumin positive GABA interneuron (blue neuron; PV+). Excessive synaptic pruning by unrestrained microglial (orange cell) elimination of dendritic spines (purple box) might be due to increased immunological signaling or immunomodulatory heterosynaptic effects of endocannabinoid neurotransmitters via the type 2 endocannabinoid receptor (CB2R). The PV+ interneurons are inhibited by cholecystokinin positive GABA interneurons (blue neuron; CCK+). Overactive CCK+ inhibition of PV+ interneurons can also lead to disinhibition of the cortical projections to the midbrain (purple neuron projecting from the orange box to the green box). The CCK+ interneurons are known to express endocannabinoid type 1 receptors (CB1R) in relatively high densities. Endocannabinoid neurotransmitters are released from the postsynaptic cell as a result of activity in G-protein coupled receptors. The endocannabinoids act on the presynaptic cell to inhibit further neurotransmitter release, and in certain circumstances, to induce long term depression of presynaptic interneuron activity. Increased inhibitory GABA signaling from CCK+ positive neurons in the context of reduced CB1R availability might result in disinhibited CCK+ inhibition of PV+ cells, pyramidal neurons, or the CCK+ cells themselves. (Pelkey et al., 2017) Additionally, the increased inhibitory tonus might lead to increased synthesis of endocannabinoids from postsynaptic membranes as suggested by increased concentrations of anandamide in the cerebrospinal fluid in schizophrenia and early psychosis. (Giuffrida et al., 2004; Koethe et al., 2009) This could contribute to dysregulation of microglial or astrocyte functions through heterosynaptic CB1R/CB2R activation.

2.7.2 The endocannabinoid system

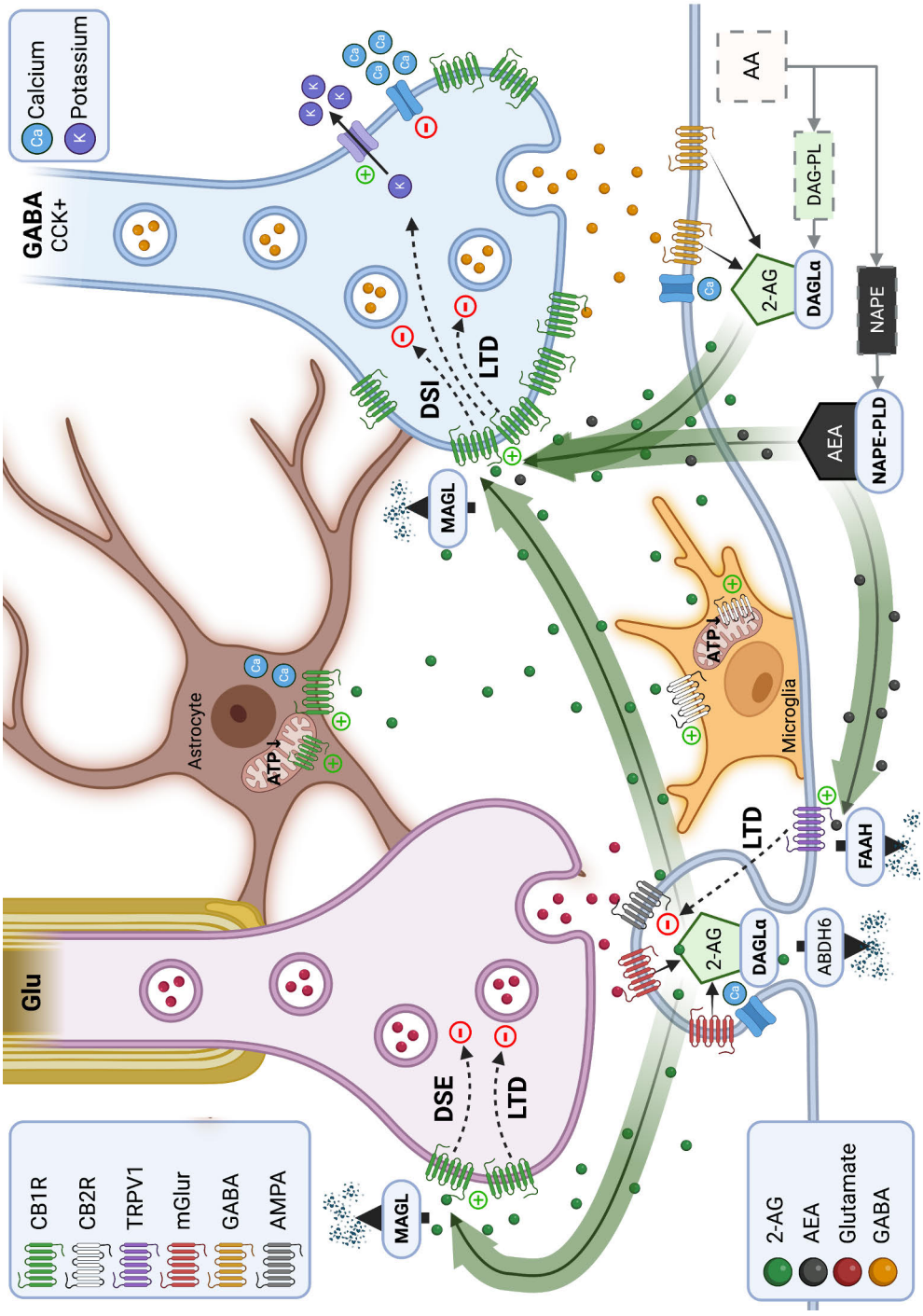
2.7.2.1 Introduction to the endocannabinoid system

The endocannabinoid system is a mode of signalling, elements of which can be found in almost all tissues of the human body. (Hillard, 2017) In the brain, the ECS functions primarily as a retrograde messenger system. (Lu & Mackie, 2016) The ECS consists of the endogenous cannabinoids, the enzymes necessary for their synthesis and metabolism, and the receptors through which the endocannabinoids act. (Lu & Mackie, 2016) The endocannabinoids i.e. anandamide (AEA) and 2-arachidonoylglycerol (2-AG), are thought to be the primary endocannabinoids regulating neural function. AEA and 2-AG exert their actions through partial and full agonism, respectively, of CB1Rs and CB2Rs. (Devane et al., 1992; Herkenham et al., 1991; Munro et al., 1993; Stella et al., 1997) The synthesis and metabolism of AEA and 2-AG are diverged as suggested by their unique pathways of synthesis and metabolism, see **Figure 3**. AEA evokes a tonic signal at the CB1R, whereas 2-AG exhibits more phasic on-demand characteristics. (Petrie, 2023) However, the concentrations of 2-AG seem to be substantially higher than those of AEA, at least in the rat brain and human CSF. (Buczynski & Parsons, 2010; Nicholson et al., 2015) In addition, the affinity of AEA for the CB1R is considerably higher than that of 2-AG: Ki≈209nM versus Ki≈3423.6nM respectively. (McPartland et al., 2009; Steffens et al., 2005) AEA and 2-AG are suggested to evoke bimodal actions on both intracellular secondary messenger systems as well as regulation of receptor internalization and expression, which would intuitively explain the evolutionary conservation of two parallel CB1R ligands, **Figure 4**. The CB1R is known to exist in an inactive, as well as an active ligand-bound conformation. (Shao et al., 2016) The active ligand-bound conformation couples to intracellular G-proteins ($G_{i/o}$); these exert their actions through inhibiting and activating second messenger systems. (Nogueras-Ortiz & Yudowski, 2016) Receptor heteromerization can however decrease receptor sensitivity for G-protein activation. (Rozenfeld et al., 2012)

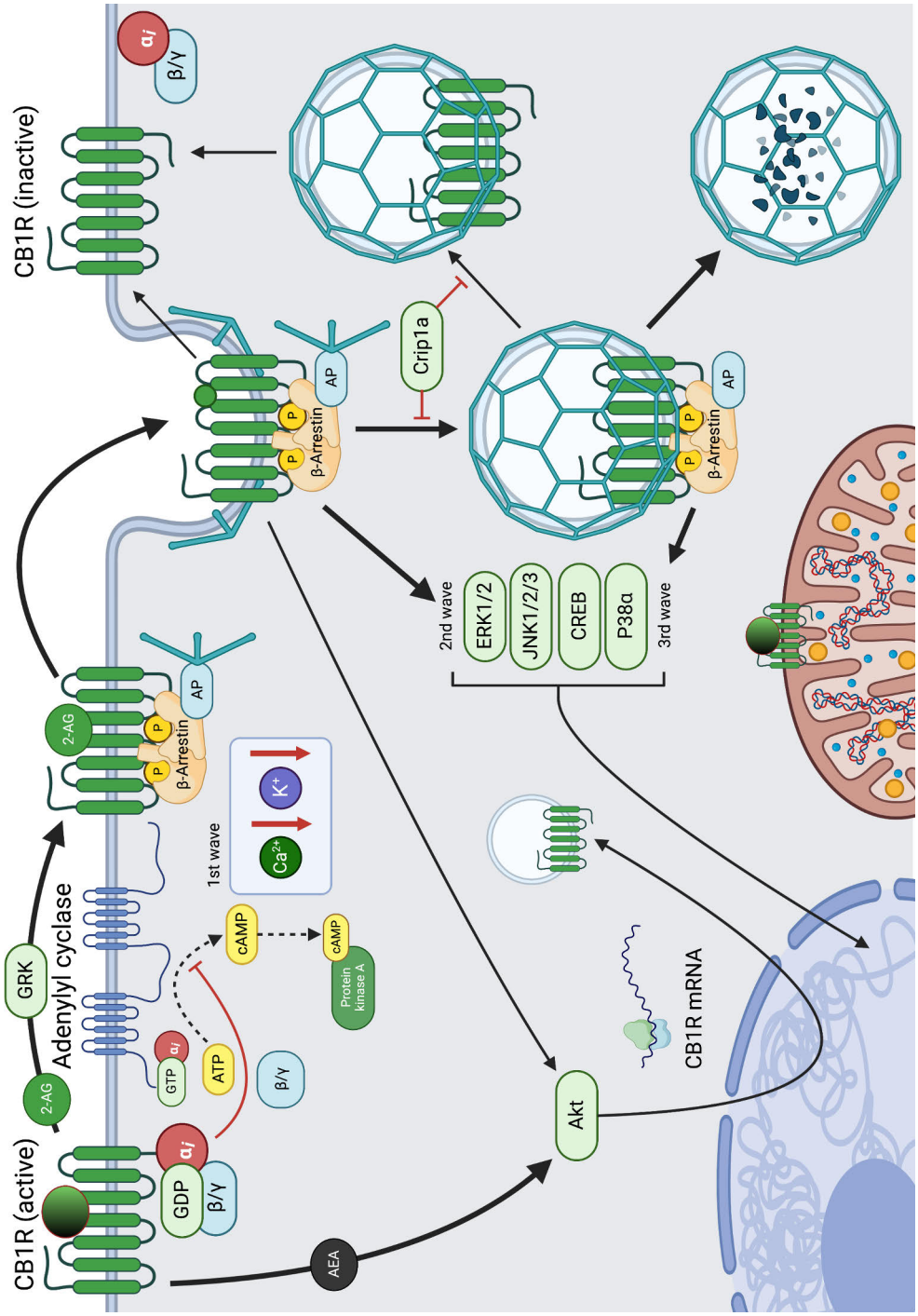
As discussed above, both CB1R and CB2R are present in the brain, but CB1R is significantly more abundant and it is the primary receptor found on neurons. (Lu & Mackie, 2016) In addition to CB1Rs and CB2Rs, the ECS has been suggested also to act through ionotropic vanilloid receptors, and nuclear hormone peroxisome proliferator-activated receptors (PPAR) to modulate membrane potentials and gene transcription respectively. (Iannotti & Vitale, 2021) While the CB1Rs are mostly found on the presynaptic plasma membranes of glutamatergic and GABAergic neurons, they can be detected on various other neurons, such as dopaminergic neurons. (Covey et al., 2017; Lu & Mackie, 2021) In the cortex, CB1Rs are most

abundant on cholecystokinin positive (CCK+) GABAergic interneurons, but also on other GABAergic interneuron types to a lesser extent. (E. L. Hill et al., 2007) The enzymatic machinery of the ECS, mode of neurotransmission and primary cellular targets of action are presented in detail in **Figure 3**. The intracellular mechanisms, regulation of the available receptor pool and bimodal multiphasic mode of action of the ECS are summarized in **Figure 4**. In macro-anatomical terms, CB1R are most prevalent within the frontal and temporal cortices, and the striatum, while they are significantly scarcer in the primary somatosensory and visual cortices, and the thalamus of healthy individuals. (Pak et al., 2023) These relative abundances of CB1Rs might reflect the high amount of CCK+ GABA interneurons in the secondary/associative cortical areas as compared to primary cortices. (Hansen et al., 2022; Whissell et al., 2015)

The ECS is involved in regulating multiple neurodevelopmental processes, (Harkany et al., 2007) short-term synaptic potentiation and long-term synaptic plasticity (Lu & Mackie, 2016), microglial responses, (Marinelli et al., 2023) and astrocyte function (Eraso-Pichot et al., 2023). As such, the ECS can influence a wide variety of higher level systems, such as endocrine functions, (Gorzalka & Dang, 2012; Micale & Drago, 2018) feeding behaviour, (Mahler et al., 2007) fear and anxiety processing, (Lutz et al., 2015) and cognition. (Kruk-Slomka et al., 2016) Providing reactions to homeostatic stress through activation of compensatory behaviours, memory consolidation and regulation of immune and hormone responses seem to be the overarching principle of the ECS at the behavioural level. The ECS has also been suggested to be involved in the pathophysiology of many psychiatric illnesses such as schizophrenia, depression and anxiety disorders. (Mustonen et al., 2021; Mustonen et al., 2018; Navarro et al., 2022) The neurodevelopmental roles of the ECS suggests that disturbances of endocannabinoid signaling during critical developmental periods, for example due to exposure to exogenous cannabinoids or adverse life experiences, (Goldstein Ferber et al., 2021; Hirvonen et al., 2012) could predispose to erroneous local circuit processing of information, (Covey & Yocky, 2021) or a large scale dysconnectivity of neural networks, (Gee et al., 2016; Sherif et al., 2016) and subsequent psychiatric symptomatology. (Fine et al., 2019)


Calcium
Potassium
GABA
CCK+
DSI
LTD
MAGL
Astrocyte
ATP
Glu
DSE
LTD
MAGL
Microglia
ATP
LTD
2-AG
DAGLα
ABDH6
FAAH
AA
DAG-PL
2-AG
DAGLα
NAPE
AEA
NAPE-PLD
CB1R
CB2R
TRPV1
mGlur
GABA
AMPA
2-AG
AEA
Glutamate
GABA

▲ **Figure 3.** An illustrated introduction to the endocannabinoid system. The endocannabinoid system is composed of the enzymatic machinery responsible for the synthesis and metabolic degradation of endocannabinoid neurotransmitters. In the lower right, the canonical synthesis of endocannabinoids occurs within the postsynaptic neuron. First, arachidonic acid (AA) is converted enzymatically through multiple enzymatic steps to either diacylglycerol (DAG-PL) or N-acylphosphatidylethanolamine (NAPE). For 2-arachidonoylglycerol (2-AG), DAG-PL is then converted into 2-AG by diacylglycerol-lipase-α (DAGLα). For anandamide (AEA), NAPE is converted into AEA by NAPE phospholipase D (NAPE-PLD). The synthesis of both AEA and 2-AG can be triggered by intracellular influx of calcium-ions (Ca) for example as a result of depolarization of the post-synaptic membrane through GABA or AMPA receptors, or activation of metabotropic glutamate receptors (mGluR) by glutamate. It is important to note that the endocannabinoids are thus synthesized on-demand after an action potential has resulted in release of neurotransmitters into the synaptic cleft. The endocannabinoids then propagate to the extracellular space where they contact the endocannabinoid receptors type 1 and 2 (CB1R and CB2R) upon which they act as agonists. The endocannabinoid receptors are G-protein coupled receptors, which are coupled primarily through $G_{i/o}$-proteins. However, other G-protein types have also been identified to couple to endocannabinoid receptors. This reflects the functional heterogeneity of these receptors. Also, heteromerization with other receptor types such as dopamine makes this system even more versatile for different populations of neurons, microglia and astrocytes. As a result of activation, the endocannabinoids can act to inhibit the further release of the contents of presynaptic neurotransmitter vesicles into the synaptic cleft, thus resulting in depolarization-dependent suppression of excitation (DSE) of glutamatergic presynaptic neurons (purple neuron; Glu), or depolarization dependent suppression of inhibition (DSI) of inhibitory GABA-neurons (blue neuron; GABA CCK+). These short-term actions result from inhibition of adenylyl cyclases, subsequent inhibition of phosphokinase A, and finally closure of voltage-gated Ca^{2+}-channels (blue ion-channel) and opening of inwardly rectifying potassium (K) channels (purple ion-channel). The latter mechanism hyperpolarizes the presynaptic neuronal membrane and suppresses further depolarizing currents. Short-term suppression of presynaptic activity can also result from metabotropic calcium influx, for example in situations where both AMPA and NMDA receptors are activated. The activity of neurons can also be suppressed by a process called endocannabinoid-mediated long-term depression (LTD), which results in an activity-independent prolonged suppression of the pre- or post-synaptic neuron. CB1Rs are also known to exert similar actions homosynaptically, and heterosynaptically to neighboring neurons. The neuron-derived endocannabinoids have also been shown to act on CB1R and CB2R residing on the cell membranes of astrocytes, microglia, and their mitochondria. The activity of the endocannabinoids is ultimately terminated by metabolizing enzymes. First the endocannabinoids are transported into the pre- or postsynaptic cell via specific endocannabinoid transporters (not shown). Both AEA and 2-AG possess unique enzymatic canonical routes for intracellular metabolism. AEA is mainly degraded by fatty-acid aminohydrolase (FAAH), while 2-AG is degraded by monoacylglycerol-lipase (MAGL). There are however other known enzymatic routes, such as alpha/beta-hydrolase domain containing 6 (ABHD6). As a whole, endocannabinoid release from postsynaptic neuron membranes has the potential to result in activity-dependent fine-tuning of further neural activity within the same synapse, inhibition of neighbouring synapses through DSI/DSE, long-lasting plasticity changes of neuronal functioning, integration of neural activity patterns into the function of microglial cells, regulation of lateral synaptic potentiation through astrocyte arrays, regulation of cellular homeostasis and energy metabolism, and as previously discussed, guiding of activated microglia migration. For more details see Eraso-Pichot et al. (2023); Lu and Mackie (2021); Marinelli et al. (2023) and references therein.


CB1R (inactive)
CB1R (active)
Adenylyl cyclase
GRK
2-AG
GDP
GTP
ATP
cAMP
cAMP
Protein kinase A
1st wave
K+
Ca2+
β-Arrestin
AP
Crip1a
2nd wave
ERK1/2
JNK1/2/3
CREB
P38α
3rd wave
AEA
Akt
CB1R mRNA

▲ **Figure 4.** The bimodal intracellular effects of CB1R binding of agonists i.e. ananadamide (AEA) or 2-arachidonoylglycerol (2-AG). The CB1R has been shown to exhibit an inactive, as well as an active ligand-bound conformation. (Manandhar et al., 2022) The inactive conformation is able to bind antagonists and inverse agonists, while the active conformation is associated with agonist binding. (Manandhar et al., 2022) Manandhar et al. (2022) also showed that molecular interactions between the ligand and amino acids of the binding pocket are different between the active and inactive conformations of CB1R, which suggests that ligand affinities can vary according to receptor conformation. AEA and 2-AG can evoke differential effects through the CB1R. (Bernabò et al., 2013; Laprairie et al., 2014) Activation of CB1R by AEA and 2-AG results in binding of $G_{i/o}$-protein complexes. (Howlett & Mukhopadhyay, 2000) These in turn inhibit the formation of cyclic adenosine monophosphate (cAMP) by adenylyl cyclase, and subsequent activation of protein kinase A. (Howlett & Mukhopadhyay, 2000) This reduces intracellular concentrations of calcium-ion (Ca^{2+}) and potassium-ion (K^{+}) resulting in hyperpolarized membrane potentials, and a reduction of the probability of Ca^{2+}-dependent release of neurotransmitters. (Fletcher-Jones et al., 2020) AEA and 2-AG differentially activate the Akt-pathway, which is suggested to trigger increased transcription of CB1R mRNA, and possibly subsequent increased CB1R protein expression. (Laprairie et al., 2014) When bound by 2-AG, CB1Rs are phosphorylated by G-protein kinase (GRK), and bound by β-arrestin-AP2-protein-clathrin-complexes. (Al-Zoubi et al., 2019; Blume et al., 2017; Manning et al., 2023; Morales et al., 2020) These promote the formation of clathrin-coated pits, which can result in the dissolution of the structure and inactivation of the CB1R protein, or internalization of the receptor protein into clathrin-coated vesicles. (Daigle et al., 2008) β-arrestin is suggested to promote the activation of various intracellular second messenger pathways such as ERK1/2, JNK1/2/3, CREB and P38α. (Dalton et al., 2020) These second messenger proteins are known to regulate the expression of a wide variety of proteins and their functions, such as immune functions and neuronal apoptosis. Signaling activity of the $G_{i/o}$-protein and β-arrestin bound receptor complexes have been shown to be present in both the clathrin-pit and intracellular vesicle state, which indicates that CB1R signaling occurs in three temporal signaling phases (1st, 2nd and 3rd wave). (Nogueras-Ortiz & Yudowski, 2016) The internalized CB1R proteins can be recycled back onto the neuronal membrane or sorted for lysosomal degradation by G protein-coupled receptor associated sorting protein 1. (Fletcher-Jones et al., 2020) A regulatory protein, the cysteine rich protein 1a (CRIP1a), inhibits the internalization of CB1R from clathrin-coated pits, but also the recycling of receptor proteins back onto the cellular membrane. (Blume et al., 2016; Blume et al., 2017; Booth et al., 2019; Smith et al., 2015)

2.7.2.2 Cannabis

Cannabis consumption has been long known to trigger psychotic symptoms, such as hallucinations and delusions. (Moreau, 1845) The effects of intravenous injection of THC in modern laboratory studies confirm these early observations. In healthy individuals, THC dose-dependently induces transient neurophysiological effects, as well as positive and cognitive symptoms, similar to those seen in schizophrenia. (D'Souza et al., 2004; Sherif et al., 2016) Epidemiological studies show that exposure to cannabis during development is linearly associated with a later risk of developing a psychotic disorder. (Marconi et al., 2016) This seems to be specifically related to the effects of THC, since the recent trend of using increasingly potent forms of cannabis has been associated with an even higher risk of psychosis compared to low-THC marijuana strains. (Hines et al., 2020) A substantial proportion (25%) of individuals with cannabis-induced psychosis later transition to fulfil the diagnostic criteria of schizophrenia. (Murrie et al., 2020) Although there is convincing evidence pointing to a causal link between cannabis use and psychosis, the reverse causation of increased cannabis use by individuals susceptible to psychotic disorders cannot be entirely ruled out. (D'Souza et al., 2022) However, international consensus guidelines suggest that persons with a known susceptibility for psychosis should probably not expose themselves to a putatively even greater health risk through exposure to cannabis. (D'Souza et al., 2022) To summarize, the endocannabinoid system is convincingly linked to the etiology of schizophrenia. The impact of endocannabinoid dysregulation might be stronger in individuals whose symptoms are mediated through the effects of exogenous cannabinoids.

The prevalence of cannabis use is increasing independently of age group and legal status of cannabis use. (Goodwin et al., 2021) Increasing population level exposure to a substance, which might increase the risk for schizophrenia, is an evident public health concern. Recent social changes have stimulated discussions about the controversies embedded in changing the legal status of cannabis use. (El-Khoury et al., 2022) We must keep in mind that the societal trends pertaining to the current cultural status of cannabis have the potential to affect scientific interpretations and hamper efforts to clarify the roles of the endogenous cannabinoid system, which exist in health and illness irrespective of exogenous cannabinoid use.

2.7.2.3 The ECS in psychotic disorders

Several lines of evidence are indicative of the presence of a disturbance in the endocannabinoid system in psychotic disorders. Concentrations of the endocannabinoid AEA are elevated in the blood and cerebrospinal fluid of psychosis

patients when compared to controls independent of whether or not the patients are using antipsychotic medications. (Garani et al., 2021; Minichino et al., 2019; Potvin et al., 2020) The concentrations of AEA in the CSF of schizophrenia patients are inversely associated with symptom severity. (Garani et al., 2021) The expression of CB1R on peripheral immune cells is also elevated independent of medication status. (Minichino et al., 2019) The blood and CSF levels of 2-AG on the other hand are unaltered in schizophrenia. (Fakhoury, 2017) However, the post-mortem concentration of 2-AG seems to be elevated in the prefrontal cortex, hippocampus and superior frontal gyrus of individuals with schizophrenia as compared to controls. (Muguruza et al., 2013; Muguruza et al., 2019; Yu et al., 2020) Interestingly, in the study conducted by Muruguza et al. (2013), the ex vivo concentrations of AEA were globally lower in schizophrenia patients in comparison to controls. The studies investigating changes in the enzymes responsible for endocannabinoid metabolism are either too few to make it possible to draw reliable conclusions on whether there are or there are no differences between individuals with schizophrenia and controls. (Garani et al., 2021)

Post-mortem studies using autoradiography of CB1R radioligands almost invariably show elevated levels of CB1R binding in the dorsolateral prefrontal cortex (Dalton et al., 2011; Dean et al., 2001; Jenko et al., 2012), the posterior cingulate cortex (Newell et al., 2006), or the anterior cingulate cortex. (Zavitsanou et al., 2004) Overall, ex vivo and in vivo studies of CB1R have produced variable results suggesting either an elevated, unchanged or decreased availability of CB1R in psychosis, see **Table 2**. To summarize, the results are difficult to interpret as hard evidence of either a lower or higher presence of CB1R in psychotic disorders as compared to controls might be due to methodological differences and sample variability. However, a disturbance of the ECS does seem to be present in both post-mortem samples of chronic schizophrenia patients, as well as in vivo samples of schizophrenia and first episode psychosis regardless of medication status. It should be noted, that the results from post-mortem samples are not entirely comparable with the results emerging from in vivo studies due to the increased number of confounders such as cumulative exposure to antipsychotic medication, somatic illness, variability in the reason of death and the post-mortem interval. Altogether, the ECS seems to be disturbed in psychotic disorders, but the variable pattern of the results due to either methodological differences between the studies, or due to the complex nature of ECS biology, makes it difficult to pinpoint the specific quality of the overall dysfunction.

Table 2. Summary of studies investigating human brain endocannabinoid type 1 receptor in psychotic disorders. The results of Study II are presented in *italics* for convenience of comparison; see chapter 5 for details.

Study	Diagnosis	n (case/control)	Method	Result	Area
Wong et al. (2010)	SCHZ	9/10	[^{11}C]OMAR PET; V_T	↑CB1R	PONS
Ceccarini et al. (2013)	SCHZ SCHZ	51/12 16*/12	[^{18}F]MK-9470 PET; mSUV [^{18}F]MK-9470 PET; mSUV	↑CB1R ↑CB1R	NA, INS, CING, FC, PAR, MTL
Ranganathan et al. (2016)	SCHZ	25/18	[^{11}C]OMAR PET; V_T	↓CB1R	AMY, CAU, PCC, HIPP, HYPO, INS
Borgan et al. (2019)	*FEP* *FEP*	*7/11* *20*/20*	*[^{18}F]FMPEP-d2 PET; V_T* *[^{11}C]MEPPEP PET; V_T*	*↓CB1R* *↓CB1R*	*HIPP, STR, ACC, THA*
Dean et al. (2001)	SCHZ	14†/14†	[^{3}H]CP-55,940 autoradiography	↑CB1R	DLPFC
Zavitsanou et al. (2004)	SCHZ	10†/9†	[^{3}H]SR141716A autoradiography	↑CB1R	ACC
Newell et al. (2006)	SCHZ	8†/8†	[^{3}H]CP-55,940 autoradiography	↑CB1R	PCC
Dalton et al. (2011)	SCHZ	37†/37†	[^{3}H]CP-55,940 autoradiography qPCR mRNA	↑CB1R n.s.	DLPFC
Jenko et al. (2012)	SCHZ	47†/43†	[^{3}H]MEPPEP autoradiography	↑CB1R	DLPFC
Deng et al. (2007)	SCHZ	8†/8†	[^{3}H]CP-55,940 autoradiography [^{3}H]SR141716A autoradiography	n.s. n.s.	STG
Eggan et al. (2008)[a] Volk et al. (2014)[a] Eggan et al. (2010)[a]	SCHZ SCHZ SCHZ SCHZ	23†/23† 12†/12† 21†/21† 14†/14†	Immunostaining; qPCR mRNA In situ hybridization [^{3}H]OMAR autoradiography Immunostaining	↓CB1R; ↓CB1R ↓CB1R ↑CB1R ↓CB1R	DLPFC
Koethe et al. (2007)	SCHZ	15†/15†	Immunostaining	n.s.	ACC
Uriguen et al. (2009)	SCHZ	11*†/11† 12*†/20† 11†/11† 7†/20†	Immunoblotting mRNA Immunoblotting mRNA	n.s. n.s. ↓CB1R n.s.	DLPFC

*: unmedicated; †: post-mortem, [a]: partially overlapping sample; ↓: lower; ↑: higher; n.s.: no significant differences between groups; FEP: first-episode psychosis; SCHZ: schizophrenia; CB1R: endocannabinoid receptor type 1; ACC: anterior cingulate cortex; AMY: amygdala; CAU: nucleus caudatum; CING: cingulate cortex; DLPFC: dorsolateral prefrontal cortex; FC: frontal cortex; HIPP: hippocampus; HYPO: hypothalamus; INS: insula; MTL: medial temporal lobe; NA: nucleus accumbens; PAR: parietal cortex; PCC: posterior cingulate cortex; STG: superior temporal cortex; STR: striatum; CB1R/CB2R agonist: [^{3}H]CP-55,940; CB1R inverse agonist: [^{3}H]MEPPEP, [^{11}C]MEPPEP, [^{3}H]OMAR, [^{3}H]SR141716A, [^{18}F]FMPEP-*d2*, [^{18}F]MK-9470.

2.7.2.4 Sex differences of the ECS

Prior evidence points to the presence of a sex difference in the ECS. For example, female rodents have decreased levels of hippocampal CB1R protein, (Reich et al., 2009) pituitary gland CB1R mRNA (González et al., 2000), CB1R/CB2R agonist [^{3}H]CP-55,940 binding, (Dow-Edwards et al., 2016; Llorente-Berzal et al., 2013; Mateos et al., 2011) when compared to males. Further, exposure to a chronic unpredictable stress, (Reich et al., 2009) or CB1R receptor stimulation, (Burston et al., 2010) produces differential responses in [^{3}H]CP-55,940 binding in male and female rodents. CP-55,940 stimulated [^{35}S]GTPγ binding, an index of CB1R function, is also dependent on the gender of the rats exposed to early developmental stress. (Llorente-Berzal et al., 2013) The direction and magnitude of CB1R sex difference in rats also seems to be dependent on the developmental phase of the non-treated animals. (Burston et al., 2010)

Human in vivo studies have shown increased levels of [^{11}C]OMAR distribution volume (V_T), (Neumeister et al., 2013; Normandin et al., 2015), and lower levels of [^{18}F]MK-9470 modified standardized uptake value (mSUV) in females in comparison to males. (Van Laere et al., 2008) The finding of higher [^{11}C]OMAR V_T in healthy females than in males has since been replicated along with results indicating that CB1R availability might differentially decrease with age depending on gender and brain area. (Radhakrishnan et al., 2022) Sex differences in the human endocannabinoid system are also theoretically supported by concepts related to the bidirectional regulation of the endocannabinoid system and gonadal hormones. (Santoro et al., 2021) See **Figure 5**.

2.7.3 The immune system in psychotic disorders

The immune system consists of a network of specialized cells, which are functionally interconnected through immune signalling. It is recognized that immune cells are dispersed throughout the body and that their functional states are orchestrated via cyto- and chemokines, such as interleukins (IL), as well as hormones and growth factors. The cells of the immune system can be categorized as capable of either innate or adaptive immune responses. Innate immune responses can be thought of as fast pre-sets of first-line non-specific responses to pathogens and antigens, while adaptive immune system responses are antigen-specific, but delayed.

The functional activation state and migration of immune cells are determined by a complex network of immune signals. These signal constellations can be simplified to drive various biological functions, such as gene expression, metabolic activity, cell proliferation and specialization, cell motility and migration, cell protection and repair as well as cell death. However, the signals are not entirely specific since they possess a considerable amount of overlapping redundancy, and they usually activate

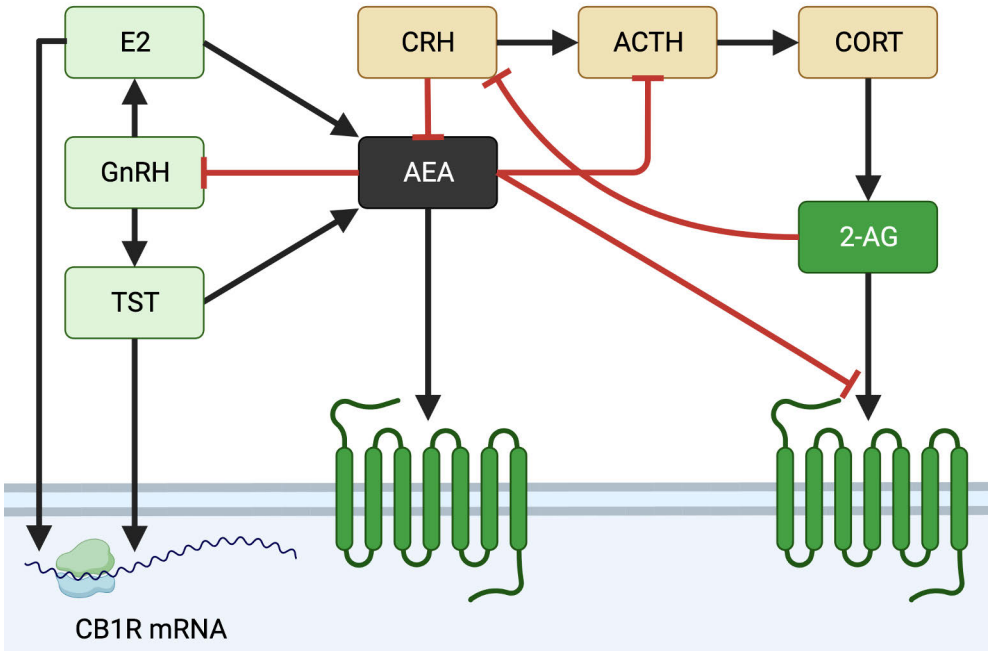


Figure 5. Hormonal interactions with the functioning of the ECS. Anandamide (AEA) signaling at amygdalar neurons projecting to the hypothalamus acts as an inhibitory gate for gonadotropic-releasing hormone (GnRH) and adrenocorticotropic hormone (ACTH) excretion from the hypothalamus. (Farkas et al., 2010; Rademacher et al., 2008) This influence inhibits the subsequent release of cortisol (CORT), estradiol (E2) and testosterone (TST) from peripheral organs. Corticotropin-releasing hormone (CRH) induces AEA metabolism, and thus releases the gate for ACTH secretion. (Hillard, 2014; Morena et al., 2016) E2 and TST act as feedback inhibitors of the gonadotropic hormonal axis by inhibiting AEA metabolism by fatty-acid amide hydrolase, and subsequent inhibition of GnRH. (M. N. Hill et al., 2007) The hormonal interplay can be considerably site specific since CRH has been shown to induce elevated 2-AG and reduced AEA levels in the rat amygdala, but not in the prefrontal cortex. (Gray et al., 2015) Stress and CORT increases synthesis of 2-AG in the amygdala, (Morena et al., 2016) which reduces the hydrolytic effect of CRH on AEA, while AEA reduces this effect by competing with the substantially lower affinity 2-AG for the orthosteric binding site at the CB1R, and through vanilloid receptor effects on intracellular signalling cascades. (Lee et al., 2015) Further, both E2 and TST can induce increased expression of CB1R mRNA and protein in a region-specific manner. (González et al., 2000; Niu et al., 2012; Riebe et al., 2010) Female rats also show a sex specific modulation of ECS mediated inhibitory signaling in the hippocampus, (Huang & Woolley, 2012) as well as increased desensitization and downregulation of CB1R secondary to stimulation with CB1R agonist THC. (Farquhar et al., 2019) These concepts point to important roles for the ECS in the sexually dimorphic physiology, and the regulation of responses to homeostatic and psychological stress. AEA seems to be the gatekeeper for stress responses, and increased 2-AG levels act to terminate the stress response. (Morena et al., 2016)

regulatory feedback-loops. Immune signalling can thus be seen to act as a complex network of push-pull signals with temporal and spatial selectivity. As a result, measuring the state of limited immune signals is not sufficient to understand the state

and direction of the immune system as a whole. Further, the multidirectional co-regulation of immune, endocrine and neural functions, all complex systems themselves, multiplies the degree of complexity of the entirety.

The innate immune system consists of macrophages, dendritic cells, basophils, eosinophils, neutrophils, mast cells and natural killer cells, as well anatomical barriers and the complement system. The microglial cell, discussed in detail below, is the main orchestrator of innate immune responses in the CNS. While the neuroimmune system is anatomically separated from the peripheral immune system by the blood brain barrier, both are however functionally coregulated through the immune signals expressed by immune cells and neuroendocrine cells.

Epidemiological studies showing an increased prevalence of autoimmune diseases in schizophrenia indicate that immunological disturbances might contribute to the pathophysiology of psychoses. (Cao et al., 2023) Peripheral blood immune signalling seems to be already altered in medication-naïve first episode psychosis patients. (Upthegrove et al., 2014) A meta-analysis conducted by Upthegrove et al. (2014) studied 570 patients and 683 healthy controls, and found significant changes in the blood concentrations of IL-1β, sIL-2r, IL-6, and TNF-α. Another meta-analysis published by Miller et al. (2011) compared the levels of peripheral cyto- and chemokines in first episode psychosis and acutely relapsed chronic schizophrenia both before and after antipsychotic treatment. They found that IL-1β, IL-6, and TGF-β showed characteristics of state, as their levels normalized after antipsychotic treatment, while IL-12, IFN-γ, TNF-α and sIL-2r levels remained elevated regardless of state or antipsychotic treatment. Yet another meta-analysis demonstrated that the CSF concentrations of IL-6 and IL-8 were elevated in both schizophrenia and major depression, and that alterations in sIL-2R, IL-1β and IL-1α were more specific to schizophrenia. (Wang & Miller, 2018) These changes in immune signalling may very well be secondary to the pathophysiology in non-immune cell types, and environmental associations. This hypothesis is supported by associations between the transcriptome and the methylome of immune system related genes, which are not consistent with the SNP associations in genome-wide association studies, except for that in the complement component 4 gene locus. (van Mierlo et al., 2020)

As reviewed above, meta-analyses of post-mortem studies on microglial involvement in schizophrenia do not wholeheartedly support the hypothesis of increased microglial density. (Snijders et al., 2021; van Kesteren et al., 2017) However, although the hypothesis of increased densities of activated microglia has not been unequivocally proven or refuted, altered peripheral immune signalling has been confirmed in meta-analyses, and radiotracers suitable for in vivo imaging of the translocator protein (TSPO) using PET in humans were developed. Thus, it seemed logical to conduct in vivo studies of microglial activation in schizophrenia, taking

into account the now dated tenet that TSPO would be a straightforward marker of increased microglial activity. (Notter, Coughlin, Sawa, et al., 2018)

TSPO density*affinity seemed to be elevated in selected studies conducted with two first-generation TSPO radiotracers, [^{11}C]PK11195 and [^{11}C]DAA1106, using binding potential as the outcome. (Marques et al., 2018) However, the overall results from this meta-analysis performed by Marques et al. (2018) which did not show changes in TSPO availability in psychotic disorders compared to controls must be viewed as inconclusive since it selectively considered studies using second generation radiotracers, or with V_T as an outcome. An updated meta-analysis reported by Plaven-Sigray et al. (2021), which did not include studies done with the radiotracer [^{11}C]PK11195 due to its arguably suboptimal signal-to-noise ratio, did indicate that the overall effect was that TSPO did display a lower availability in patients with psychotic disorders in comparison to controls.

2.7.4 Brain glial cell function in psychotic disorders

2.7.4.1 Neurodevelopmental origins

The human central nervous system (CNS) develops out of a specialized part of the outermost germ layer of the embryo called the neuroectoderm. This layer forms the neural plate, which doubles up into the neural tube, whose cephalic end later expands tremendously, repeatedly folds onto itself, and thus forms the convoluted mass we know as the brain. During these developmental changes, the brain regionalizes into three functionally separable parts: the rhombencephalon, mesencephalon and the telencephalon. The latter further expands to form the diencephalon, and the cerebral hemispheres, which house the cerebral cortex. The cells of the brain are mostly either neurons or glial cells; the approximate proportions are: 50% of cells are neurons, 25% oligodendrocytes, 20% astrocytes, 5–15% microglia and 3–10% are oligodendrocyte precursors. (Allen & Lyons, 2018) These non-neuronal cells have specialized out of neuroectodermal germ cells with one exception: microglia become embedded in the nervous system during embryonic development, and they are thought to originate from yolk sac progenitor cells, rather than neuroectodermal progenitors. (Ginhoux & Prinz, 2015) The presence of microglia is vital for the post-natal developmental processes occurring within the brain. (Wolf et al., 2017) Their immunological origin dictates that their actions subject the nervous system to regulation by the immune system. Meanwhile, the progenitor cell derived neurons and glia migrate to their destinations according to chemo-attractive gradients. The neurons then grow axons towards their targets to form the backbone of the connectivity of the brain. An explosion of synaptogenesis starts in utero, and it continues into the early post-natal period. (Huttenlocher & Dabholkar, 1997) A

ruthless competition for existence then ensues, and it continues until early adulthood. This competition results in the elimination of redundant inactive synapses at least partly through synaptic pruning conducted by microglia. (Wolf et al., 2017) These processes are orchestrated by a balance of a multitude factors, and they can potentially result in undesirable consequences, for example dysconnectivity may be the result when synaptic pruning is excessive. For more details on this paragraph, see Kandel (2021) and references therein unless otherwise indicated.

2.7.4.2 Microglia

Microglia are the tissue-resident macrophages of the brain. They are part of the mononuclear phagocyte system along with other tissue macrophages, dendritic cells, and monocyte-derived CNS cells. As cells of myeloid origin, the microglia express immune markers, and exhibit immune functions similar to their peripheral equivalents. They are evenly dispersed throughout the brain parenchyma, as opposed to other cells of myeloid origin, which migrate mainly to the perivascular and -meningeal spaces of the brain. Each microglia cell occupies a specific territory of brain parenchyma through which it protrudes elongated appendixes. It can thus monitor the chemical environment, and exert actions within the spaces cohabited by neurons and other glial cells. (Kettenmann et al., 2011; Prinz & Priller, 2014)

The functional states of the microglia can be divided into three forms: 1) a surveying state (M0), 2) a proinflammatory state (M1), or 3) an anti-inflammatory state (M2). It should be noted however that this categorization is most likely an oversimplification. The ubiquitous presence of the microglia in various environments, composed of different neuron types, glial cell compositions and extracellular chemical gradients, dictates that the phenotypes of activated microglia are heterogenous, and can vary flexibly between the extreme phenotypes of "M1" or "M2". (Stogsdill et al., 2022) Furthermore, the properties of both of the activated phenotypes can co-exist in a single microglial cell. (Kettenmann et al., 2011) Nonetheless, the simplified phenotypes are still useful for demonstrating the various types of microglial functions. For an illustrated introduction into the roles and functions of the microglia, see **Figure 6**.

Epidemiological and genetic associations, (Benros et al., 2014; Pouget et al., 2019; Ripke et al., 2014; Sekar et al., 2016; van Mierlo et al., 2020), as well as changes in various immune markers measured from central and peripheral tissues, (Gandal, Zhang, et al., 2018; Upthegrove et al., 2014; Wang & Miller, 2018) are evidence for an etiological role for the immune system in schizophrenia. Consequently, while microglia have been suggested to be increased in number and/or activation state in schizophrenia, which could in turn lead to expressive pruning of

synapses during development (Howes & McCutcheon, 2017), the evidence base behind this suggestion is however currently lacking.

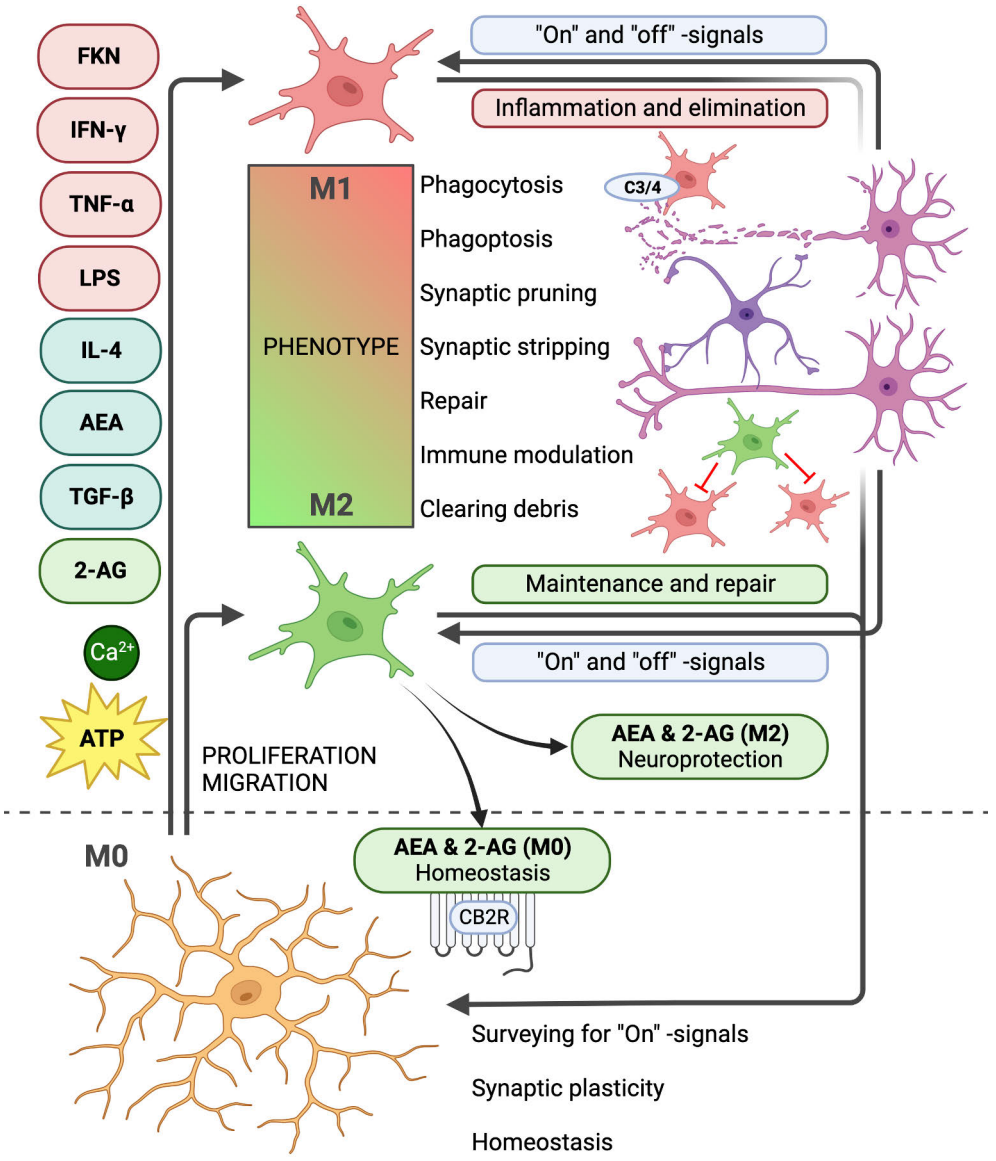
Post-mortem studies using a variety of immuno- or radiolabels have shown increased or similar levels of microglia staining/binding in individuals with schizophrenia in comparison to controls. (Laskaris et al., 2016) Study heterogeneity, such as differences in sample characteristics, post-mortem interval, studied area, label used and general methods, may explain some of the variance in these study results. In one recent meta-analysis of substantially heterogenous post-mortem studies, with partially overlapping samples, the densities of microglia were higher in patients with psychoses than in control subjects, while astrocyte densities remained unchanged. (van Kesteren et al., 2017) Contradictory to this, the expression of chemokine receptor gene CX3CR1, which is associated with the pro-inflammatory phenotypes of microglia, was previously shown to be decreased in the brain of individuals with schizophrenia in comparison to controls. (Bergon et al., 2015) Further studies have also demonstrated that CX3CR1 and other microglia associated genes are down-regulated in patients with schizophrenia. (Gandal, Haney, et al., 2018; Gandal, Zhang, et al., 2018) A decrease in CX3CR1 expression is interesting as a potential biomarker, since this protein is expressed exclusively by microglia within the brain. (Schulz et al., 2012) An updated meta-analysis conducted by Snijders et al. (2021) of studies investigating post-mortem microglial density addressed some of the problems of heterogeneity and data overlap present in the publication of van Kesteren et al. (2017). The results of this refined meta-analysis contradicted the results of van Kesteren et al. (2017): overall microglial density and morphology were unchanged in schizophrenia when compared to controls. Further, this new meta-analysis replicated the results of genome-wide transcriptome studies of decreased CX3CR1 expression, in addition to decreased expression of other microglia related genes. (Berdenis van Berlekom et al., 2020) Somewhat confusingly in light of the previously discussed results, CX3CR1 signalling seemed also to be neuroprotective as shown in results emerging from tests done in CX3CR1 knockout mouse models. (Cardona et al., 2006) Fractalkine-CX3CR1-signalling has been associated with the regulation of microglial phenotypes, microglial migration, and also microglial regulation of synaptic structures and function. (Camacho-Hernandez & Pena-Ortega, 2023) CX3CR1 deficient microglia seem to be lacking the capability to provide neurotrophic support to neurons, which could lead to their pivoting towards unrestricted actions associated with pro-inflammatory phenotypes. As suggested above, while the reduced expression of genes associated with the migration and activation of microglia does not support the concept of increased densities of active microglia, it should be recalled that the regulation of microglial phenotypes is rather complex, **Figure 6**. For example, a disruption of fractalkine-CX3CR1-signalling has been suggested to be neuroprotective in some neurological

states, but pro-inflammatory in others. (Pawelec et al., 2020) Silence within one activating regulatory signal route could lead to an overemphasis of other functions; their putative benefits could depend on the type of insult or affected neuron population. The heterogeneity of microglial phenotypes, and the possibility of co-existing pro- and anti-inflammatory stances, suggests that microglia related pathological phenomena, such as excessive loss of dendritic spines, could potentially occur without any observable increase in the densities of active microglia.

Observations of markedly increased translocator protein (TSPO) expression in states associated with activated microglia, (Sandiego et al., 2015) and the development of adequate TSPO radiotracers, (Collste et al., 2016; Turkheimer et al., 2015) led to a series of in vivo human PET studies investigating microglial function in psychotic disorders. (Marques et al., 2018; Plaven-Sigray et al., 2021) TSPO is discussed in more detail below, but for the purpose of this topic it should be mentioned that the overall impression emerging from the studies using second generation radiotracers was that of a decreased TSPO availability in subjects with psychotic disorders in comparison to controls. (Plaven-Sigray et al., 2021) Even though the hypothesis of increased densities of active microglia in schizophrenia seems increasingly unlikely in the light of the evolving evidence base, the post-synaptic spines of neurons do seem to be reduced in schizophrenia when compared to healthy individuals, and microglia remain as one of the suspected culprits behind this phenomenon. (Berdenis van Berlekom et al., 2020) It would be beneficial to understand the regulatory forces underpinning the functional balance of activated microglia, given that TSPO upregulation is selective for microglial cells in the pro-inflammatory state, as opposed to microglial cells which express the characteristics of other phenotypes. (Pannell et al., 2020)

The microglia exist in a constant interaction with neurons via a plethora of molecular signals. These modulate the mode of control evoked by microglia on synaptic structure and function. (Marinelli et al., 2019) For example, endocannabinoids act on microglial endocannabinoid receptors and ionotropic vanilloid receptors to promote the "M2" neuroprotective phenotype, or the "M0" homeostatic phenotype. (Marinelli et al., 2023; Tanaka et al., 2020)

Given the complexity of microglial activity and phenotypic regulation, and their critical roles in maintaining brain health as described in **Figure 6**, it is conceivable that a shift in peripheral immune signalling, developmentally primed microglia, and/or altered neurotransmitter systems could also lead to an excessive loss of synapses as a result of microglia underperforming their neuroprotective "M2" roles. Understanding the precise quality of microglial dysfunction would be of the utmost importance if one were to try to develop new therapeutics targeting microglia, since silencing one type of microglial function altogether might counterproductively overemphasize the functions of the others.


FKN
IFN-γ
TNF-α
LPS
IL-4
AEA
TGF-β
2-AG
Ca2+
ATP
"On" and "off" -signals
Inflammation and elimination
M1
PHENOTYPE
M2
Phagocytosis
Phagoptosis
Synaptic pruning
Synaptic stripping
Repair
Immune modulation
Clearing debris
C3/4
Maintenance and repair
"On" and "off" -signals
AEA & 2-AG (M2)
Neuroprotection
PROLIFERATION
MIGRATION
M0
AEA & 2-AG (M0)
Homeostasis
CB2R
Surveying for "On" -signals
Synaptic plasticity
Homeostasis

◄ **Figure 6.** In their resting state (M0; orange cell), the microglia are committed to specific territories, whose environment they survey for proinflammatory signals, and markers of pathogens. **The M0 microglia** protrude thin elongations into the brain parenchyma, through which they are able to monitor it's chemical environment without needlessly interfering with neuronal function. The M0 microglia have also been suggested to support neuronal homeostasis, and regulate synaptic plasticity. When the M0 microglia detect an activating "On"-signal, their morphology changes to resemble a more amoebic form, and they start to migrate towards the origin of the signal guided by chemical gradients. The change in morphology is necessary to facilitate navigation through the tightly packed parenchyma. "On"-signals, such as extracellular bursts of calcium Ca^{2+}, and excessive release of adenosine triphosphate (ATP), have been shown to trigger the change to an active morphology. Other chemical signals, such as the endocannabinoid 2-arachidonoylglycerol (2-AG), guide the microglia to their destination. Note however, that the microglia also possess receptors for other neurotransmitters such as glutamate and GABA, which can modulate the phenotype and/or activity status of the microglia. At their destination the microglia can assert a variety of functions. Chemical signals, such as fractalkine (FKN), bacterial lipopolysaccharides (LPS), tumor necrosis factor alpha (TNFα) and interferon gamma (IFNγ), pivot the microglia towards the proinflammatory (M1; red cell) state. **The M1 microglia** can be seen as proinflammatory agents who eliminate cells labelled as unwanted, and orchestrate the actions of other inflammatory immune cells through chemokines (e.g. C-C motif chemokine 22). For example, the microglia can phagocytose dying neurons, or induce the death of, and phagocytose, neurons expressing apoptotic signals (phagoptosis). They have also been shown to eliminate synapses (synaptic pruning) labeled by complement factor 3 and 4 (C3/4). As a milder version of synaptic pruning, the microglia can strip the inhibitory synapses (synaptic stripping) of parvalbumin expressing inhibitory interneurons from their glutamatergic excitatory counterparts. This regulation of synaptic connectivity is especially pertinent to the glutamate and neurodevelopmental theories of schizophrenia discussed above. On the other hand, other signals such as interleukin (IL) 4, anandamide (AEA) or transforming growth factor beta (TGFβ), pressure the microglia to a more anti-inflammatory phenotype (M2; green cell). **The M2 microglia** act according to homeostatic signals to repair compromised structures and to maintain the optimal environment for neuronal functioning. They also clean the extracellular space of debris, which could potentially interfere with the functioning of neurons. As mentioned above, the functional balance of microglial phenotypes is pertinent to determining neurodevelopmental trajectories. The long life-span of the microglia is also to be considered concerning development. Powerful early environmental insults have a propensity to affect the regulation of microglial functioning well after the adverse event. Primed microglial cells could be shifted towards pro-inflammatory states, which would correspondingly translate into excessive synaptic pruning, elimination of neuron progenitors, reduced anti-inflammatory modulation, and stripping of inhibitory synapses from cortical projections to the midbrain. The 'multi hit' -theory of schizophrenia posits that a sequence of adverse events is necessary for the biological changes of the disease to emerge. (Davis et al., 2016) Developmentally primed microglia, genetically determined synaptic dysfunction, and an assembly of nefarious environmental stress factors could, in theory, explain the biological changes seen in schizophrenia. For example, the endocannabinoid system, which has a role in regulating microglial function and location, is certainly amenable to developmental priming. (Goldstein Ferber et al., 2021; Meyer et al., 2018) The two endocannabinoids, anandamide (AEA) and 2-AG, are released from neurons and microglial cells. Both have been suggested to play a dual role of attracting microglial migration, and promoting anti-inflammatory microglial genotypes M2 and M0 via acting through the endocannabinoid receptor type 2 (CB2R). (Marinelli et al., 2023; Tanaka et al., 2020) Finally, neurons and astrocytes are able to modulate microglial phenotypes and activity at their migration destinations by local chemical signals ("On" or "off" -signals). The microglia can revert back to the quiescent M0 phenotype after a phase of activation. Unless indicated elsewhere, see Kettenmann et al. (2011) for references concerning microglial physiology, and Brown and Neher (2014) with respect to microglial cytotoxicity and synaptic modulation.

2.7.4.3 Astrocytes

Astrocytes are roughly star-shaped glial cells, which interact functionally with thousands of neurons and other cell types of the brain through extensive radial arborisations. They interact with developing neurons to form the functional unit known as the tripartite synapse. (Araque et al., 1999) In fact, it has been shown that mature neurons are not viable without developing alongside their astrocyte counterparts, and that the normal functioning of astrocytes is absolutely necessary for the formation of functioning synapses, and thus for neurodevelopment as a whole. (Farhy-Tselnicker & Allen, 2018)

The astrocytes are electrically non-excitable cells; they monitor the synaptic environment for chemical signals, such as increases in Ca^{2+} concentrations, and respond in turn by releasing their own regulatory signals, called gliotransmitters, to modulate neuronal function. (Araque et al., 1999) These mechanisms are largely regulated by intracellular fluctuations of Ca^{2+} concentrations, which are in turn affected by voltage-gated Ca^{2+} -channels or Ca^{2+}/Na^{+}-exchangers, and buffered by intracellular organelles such as the endoplasmic reticulum and mitochondria. (Eraso-Pichot et al., 2023)

Synaptic activity induces Ca^{2+} transients in astrocytes, which in turn triggers the release of gliotransmitters, such as glutamate or GABA. Regional astrocytes are interconnected, and through these interconnections, the Ca^{2+} transients are able to propagate to neighbouring astrocytes in order to spatially and temporally integrate neural signals. Thus, the astrocytes release gliotransmitters as an array to modulate the synaptic neurotransmission of a group of neurons, regardless of where the original activity took place, **Figure 7**. (Eraso-Pichot et al., 2023; Schipke et al., 2008)

In addition to their roles in neurodevelopmental processes and as coordinators of short-term synaptic activity patterns, the astroglia function as regulators of synaptic plasticity, inflammation, extracellular ion homeostasis and metabolism. (Dallérac et al., 2018)

Astrocyte pathology has been suspected to contribute to the complex deficits seen in schizophrenia. (de Oliveira Figueiredo et al., 2022) Although the post-mortem evidence of astrocyte involvement in schizophrenia is just as inconclusive as the evidence for a microglial involvement, in vitro studies of glial cell precursors do suggest there may be disrupted transcription of genes relevant for the specialization of developing astrocytes, and functioning of mature astrocytes. (Szabo et al., 2021; Windrem et al., 2017) The neurodevelopmental functions of astrocytes would imply that they could contribute to the pathology of schizophrenia as suggested by the neurodevelopmental theory. However, astrocytes have also been shown to regulate glutamate and dopamine neurotransmission (de Oliveira Figueiredo et al., 2022) and these cells have also been speculated to modulate inflammatory responses through the regulation of extracellular endocannabinoid

concentrations, since they express the enzymes necessary for the synthesis and metabolism of endocannabinoids. However, the presence and precise role of putative endocannabinoid release from astrocytes are currently unclear. (Eraso-Pichot et al., 2023)

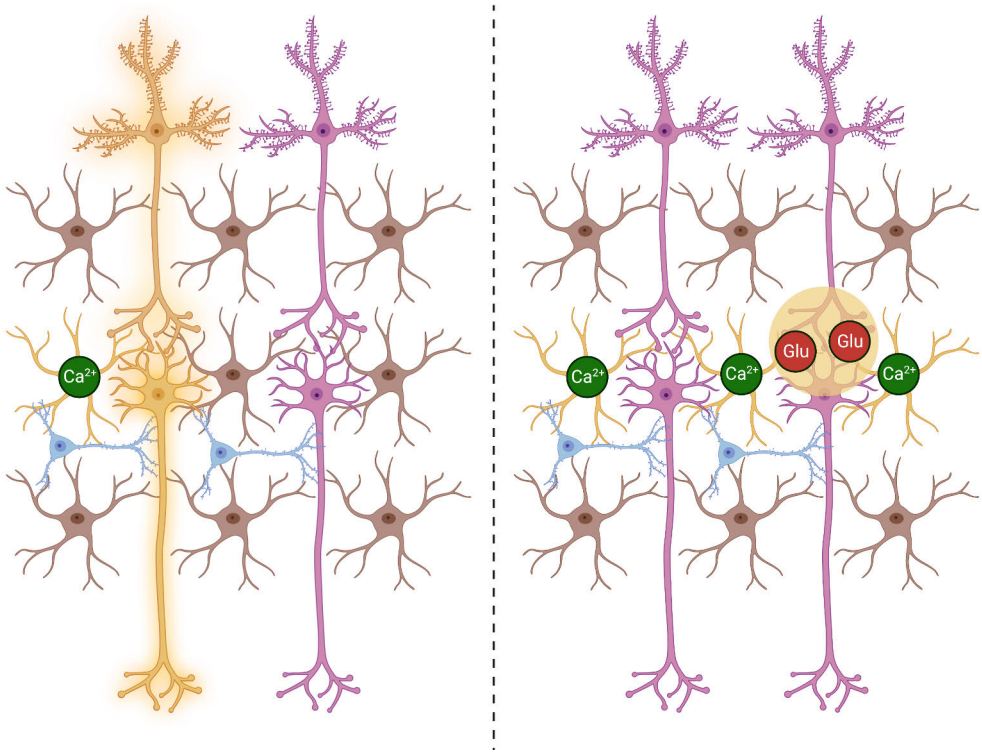


Figure 7. Short-term modulation of synaptic activity by astrocytes. On the left, an array of astrocytes (brown cells) is connected to thousands of neurons (purple cells) through extensive arborization. The signals integrated into an action potential (yellow neuron) are chemically propagated from one neuron to another via the synapse. The subsequent postsynaptic release of endocannabinoids heterosynaptically activates CB1R on astrocytes, which in turn leads to increased intracellular calcium levels (yellow astrocyte; Ca^{2+}). On the right, transient elevations in calcium concentrations travel throughout the array of astrocytes connected physically by gap junctions. As a result of these calcium transients, the gliotransmitter glutamate (Glu) is released to act on nearby neurons. These neurons are thus sensitized (yellow circle) as a result of lateral synaptic potentiation through astrocyte gliotransmission. This is an example of how astrocytes spatially and temporally integrate signals into activity patterns of a large population of neurons. For references see Schipke et al. (2008) and Eraso-Pichot et al. (2023).

Endocannabinoids have been convincingly demonstrated to affect astrocyte function. For example, the CB1R is present in the plasma membrane of astrocytes and these receptors are also expressed on the membranes of certain intracellular organelles such as the mitochondria. Endocannabinoids released from postsynaptic

neuronal membranes diffuse to adjacent astrocytes, and act on CB1Rs causing transient intracellular increases in the Ca^{2+} concentration. As intracellular Ca^{2+} concentrations are integral for the regulation of how astrocytes respond to extracellular signals, the endocannabinoid system can modulate a wide variety of these functions, such as lateral synaptic potentiation and gliotransmitter release, **Figure 7**, as well as the regulation of astrocyte metabolism through mitochondrial CB1Rs. Interestingly, the propagation of Ca^{2+} dependent lateral synaptic potentiation also seems to be dependent on mitochondrial CB1R signalling. Ionotropic vanilloid receptors, and nuclear hormone PPAR receptors have also been implicated as regulators of astrocyte responses to endocannabinoid signalling. Furthermore, evidence for a CB1R allosteric modulation by gonadal hormones in astrocytes suggests a deep-rooted connection to the endocannabinoid system. In addition, TSPO availability as measured by [^{18}F]FEPPA and PET seems to be increased in the context of long term use of exogenous CB1R partial agonist THC. (Da Silva et al., 2019) This further supports the idea that these systems are interconnected, since TSPO is expressed primarily in the mitochondria of microglia and astrocytes in the brain (Notter, Coughlin, Sawa, et al., 2018). For more details on the information described in this paragraph see Eraso-Pichot et al. (2023) unless indicated otherwise.

It could be argued that endocannabinoid dysregulation of astrocytes could contribute to the developmental pathologies underpinning psychotic disorders given the critical role of astrocytes in neurodevelopmental processes. In addition, endocannabinoid dysregulation might disrupt the spatial and temporal fine-tuning of neuronal arrays, which could in turn lead to aberrant local network processing of information.

2.7.5 The translocator protein

TSPO is an evolutionally well-conserved 18kDa molecule, which is expressed primarily on the outer membrane of mitochondria. It has been speculated that during the course of evolution, the roles of TSPO have changed from an environmental sensor to intracellular space sensor after the mitochondria relocated inside mammal cells. Although archaic in origin, the TSPO is not entirely irreplaceable for cellular function, as some species of plants and yeast do not possess homologue genes for TSPO. While several lipids, proteins, porphyrins and peptides can bind to TSPO, no specific endogenous TSPO ligand has been identified. TSPO has been associated with important cellular functions such as steroidogenesis and the regulation of cellular respiration. In cells of the nervous system, TSPO has also been suggested to regulate the expression of mitochondrial membrane potentials (MMP; an index of the bioenergetic state of mitochondria), voltage-dependent anion channels (VDAC)

and cytosolic Ca^{2+} concentrations. For more details, see Lee et al. (2020) and references therein.

As previously discussed, CB1Rs are also expressed on the outer membrane of mitochondria, where they participate in regulating mitochondrial cellular respiration in response to endocannabinoid stimulation. (Benard et al., 2012) which links endocannabinoid signalling to the energy metabolism of CNS cells, and possibly to the other mitochondrial functions discussed above. Interestingly, treatment of transgenic Alzheimer's disease model mice with a PPARγ agonist led to significantly decreased [^{3}H]PK11195 binding to TSPO compared to non-treated mice. (Roberts et al., 2009) Endocannabinoids have been shown to act at PPARs resulting in a modulation of gene transcription of the target cell. (Iannotti & Vitale, 2021)

In vitro human microglial TSPO gene knockout studies have provided more insights into the implications of altered TSPO expression. Instead of affecting steroidogenesis, TSPO deletion reduced the intensities of MMPs, and increased cytosolic Ca^{2+} concentrations in microglia. (Milenkovic et al., 2019) Furthermore, at the level of cellular function, microglia isolated from TSPO knockout mice seemed to exhibit a reduced phenotypic activation secondary to IL-4 and LPS stimulation when compared to microglia from wildtype animals. Microglia without TSPO exhibited lower mitochondrial levels of oxidative phosphorylation and glycolysis, which are signs of disturbed cellular metabolism. (Yao et al., 2020) In another in vitro TSPO knockout study, the TSPO deficient microglia similarly exhibited attenuated responses to pro-inflammatory signals, altered metabolism, increased mitochondrial Ca^{2+} concentrations, as well as reduced VDACs and MMPs. (Bader et al., 2023) TSPO knockout animals are however surprisingly viable considering the crucial metabolic functions regulated by TSPO. (Banati et al., 2014) Notably, although mitochondrial metabolism was similarly reduced in the TSPO knockout mice, they exhibited similar microglial activation-related secondary responses to an insult as wild-type mice, which is at odds with the results obtained in the in vitro studies. (Banati et al., 2014)

In humans, TSPO has also been implicated as a regulator of steroidogenesis. The rs6971 Ala147/Ala polymorphism in the TSPO gene was associated with reduced plasma corticosteroid responses in comparison to the values from individuals with Thr147/Thr genotype. (Owen et al., 2017) Conclusions about the CNS effects cannot however be drawn from peripheral measures since animal studies have suggested that steroidogenesis is unaltered in the brains of TSPO deficient animals. (Lee et al., 2020)

There is a wealth of knowledge indicating that TSPO expression is greatly increased in various inflammatory states of the brain. Animal disease models have been used to validate the assay of TSPO as a biomarker of inflammation in various CNS pathological states. Studies conducted in humans have detected increased

TSPO expression in neurological states such as brain injury, glioblastoma, multiple sclerosis and Alzheimer's disease. However, the expression of TSPO seems to be dependent on the type of immunological insult, since also lower TSPO levels have been reported as a response to selected neuropathology. For example, low levels of irradiation (0.01Gy) resulted in acutely decreased levels of mitochondrial TSPO in mice, (Betlazar et al., 2021) possibly as a result of activation of neuroprotective "M2" microglia, i.e. it seems that the expression of TSPO varies depending on the cellular environment or type of insult. (Beckers et al., 2018; Bonsack et al., 2016) Though primarily considered to be a marker of microglial activation, TSPO has subsequently been shown to be expressed in considerable amounts also in astrocytes and endothelial cells, and to a lesser extent in neurons. Although useful as a biomarker of states of robust neuroinflammation, the temporal profile and dynamics of TSPO expression in low-level inflammation are not well understood. The ambiguous origin of human TSPO measured in vivo adds to the problem, since putative changes cannot be attributed to a definite cellular source. For more details on the information described in this paragraph, see Guilarte et al. (2022) and references therein unless otherwise indicated.

2.8 [^{18}F]FMPEP-*d2*, [^{11}C]MEPPEP and human in vivo PET imaging

The radioligands [^{18}F]FMPEP-*d2* ((3R,5R)-5-[(3-fluoromethoxy-d2)phenyl]-3-[(R)-1-phenylethylamino]-1-(4-trifluoromethylphenyl)pyrrolidin-2-one) and [^{11}C]MEPPEP ((3R,5R)-5-(3-methoxy-phenyl)-3-((R)-1-phenylethylamino)-1-(4-trifluoromethylphenyl)pyrrolidin-2-one) can be utilized to study the CB1R with PET. (Donohue et al., 2008) Similar to rimonabant, they are both CB1R selective inverse agonists at the orthosteric binding site for CB1R. Both compounds have been shown to have high lipophilicity and excellent uptake into the human brain. (Terry, Hirvonen, Liow, Seneca, et al., 2010) The relevant radiotracer characteristics of [^{18}F]FMPEP-*d2* and [^{11}C]MEPPEP are presented in **Table 3**. This comparison reveals that although approximately similar in brain uptake and affinity parameters, the intra-class correlation coefficient (ICC) of the area-under-curve of plasma activity of [^{11}C]MEPPEP is poor due to high intersubject and retest variabilities. (Terry, Hirvonen, Liow, Zoghbi, et al., 2010) This probably translates into the higher intersubject variability of [^{11}C]MEPPEP V_T, since the brain uptake parameters of both ligands are rather comparable. Nonetheless, the ICC of [^{11}C]MEPPEP V_T has been found to be good. Both tracers can be similarly quantified using 2-tissue compartment modelling (2TCM) as shown in **Table 3**. (Terry, Hirvonen, Liow, Zoghbi, et al., 2010) A graphical analysis by a Logan-plot produced comparable retest variabilities and ICCs of V_T as compared to 2TCM (correspondingly,

depending on the area: 9–17% and 0.85–0.91 for 2TCM; 13–18% and 0.85–0.93 for Logan-plot), when studied in healthy humans. (Zanotti-Fregonara et al., 2013)

Table 3. Comparison of [^{11}C]MEPPEP and [^{18}F]FMPEP-*d2*. Table adapted from (Terry, Hirvonen, Liow, Zoghbi, et al., 2010) with the permission of the corresponding author.

	[^{11}C]MEPPEP	[^{18}F]FMPEP-*d2*
K_i (nM)*	9.6 ± 0.1	9.6 ± 0.1
Methandamide K_b (nM)**	0.47	0.19
CB1R vs. CB2R selectivity**	769	3580
Brain uptake		
Peak in putamen (SUV)	3-4	3-4
% of peak after 2 hours	~80%	~70%
Inter-subject variability	16%	14%
Retest variability	8%	16%
ICC	0.77	0.33
Distribution volume		
V_T (mL•cm^{-3})	12-29	13-24
SE (%)	3-7	1-3
Inter-subject variability	>50%	26%
Retest variability	15%	14%
ICC	0.87	0.89
Specific V_T (striatum; pons)	88%; 70%	85%; 58%
Plasma $AUC_{0-\infty}$		
Inter-subject variability	>200%	13%
Retest variability	58%	16%
ICC	-0.02	0.80

The data for [^{11}C]MEPPEP are derived from 17 healthy subjects, 8 of whom had retest scans (Terry et al., 2009). The data for the [^{18}F]FMPEP-*d2* are derived from 9 healthy subjects, 8 of whom had retest scans. (Terry, Hirvonen, Liow, Zoghbi, et al., 2010) * Unpublished data; measured in human cerebellum; ** (Donohue et al., 2008)

2.9 [^{11}C]PBR28 human in vivo PET imaging

The second generation specific TSPO radiotracer [O-methyl-^{11}C]N-acetyl-N-(2-methoxybenzyl)-2-phenoxy-5-pyridinamine ([^{11}C]PBR28) was developed to provide improved tracer characteristics to avoid the low signal-to-noise ratio and high non-specific binding associated with [^{11}C]PK11195. (Imaizumi et al., 2008). However, the improved specific binding introduced a new problem in that it revealed a common polymorphism in the TSPO gene (rs6971), which affected the binding site for [^{11}C]PBR28, and probably also for [^{11}C]PK11195, although in the latter case, the

compound's poor specific binding had masked the effect attributable to the genotype. (Kreisl et al., 2010; Owen et al., 2012) The radiotracers did not exhibit any binding to TSPO if the gene was homogenous for the Thr147/Thr single nucleotide polymorphism (low affinity binding genotype; LAB), and exhibited mediocre binding in heterogenous Ala147/Thr genotypes (medium affinity binding genotype; MAB), and only full binding affinity in the Ala147/Ala genotype (high affinity binding genotype; HAB). (Owen et al., 2012) This added an unwanted complexity to PET imaging studies, since it was necessary to determine the TSPO rs6971 genotype in each participant in order to exclude LABs with no binding, and to account for the ~65% higher V_T of HAB compared to MAB. (Collste et al., 2016) Otherwise, [^{11}C]PBR28 exhibited good characteristics for brain imaging. The affinity of [^{11}C]PBR28 for TSPO was K_i=0.58±0.25 nM, i.e. ~6 times higher than that of [^{11}C]PK11195. The other radiotracer properties and references are presented in **Table 4**. Results from a test-retest study performed by Collste et al. (2016) suggested that V_T might be subject to diurnal variation. The same study examined the suitability of different kinetic models, and PET acquisition time, with the result that 2TCM modelling produced the lowest absolute variability in the test-retest setting, and that reducing the acquisition time from 91 minutes to 63 minutes did not increase the absolute variability of V_T.

Table 4. Key characteristics of [^{11}C]PBR28

	[^{11}C]PBR28
K_i (nM)*	0.58 ± 0.25
Brain uptake	
Peak uptake (SUV)	3–5
Peak uptake time	40min
Absolute variability	14.6%
Distribution volume (2TCM)	
V_T (mL•cm^{-3})	1.3–7.7
SD	1.7
Absolute variability	16.9%
ICC	0.9–0.94
Specific V_T*	>95%
Plasma	
Absolute variability ($AUC_{0-\infty}$)	17.4%
Plasma free fraction	5.2–13.8%

The data are derived from 12 healthy subjects measured twice on either the same day (n=6), or on separate days (n=6), (Collste et al., 2016) except for * (Imaizumi et al., 2008)

3 Aims

The purposes of this thesis were to examine the hypothesis that pathology in the endocannabinoid system, glial cells, and immune system is present in transdiagnostic samples of first episode psychosis, and to study theoretically how they associate to the dopamine theory of schizophrenia. It should be stated that the research projects which funded the materials for this thesis took place in 2009/2013 for the endocannabinoid studies ("CB1-PET-2009"/"METSY"), and 2013 for the studies of peripheral immunology and glial cell function ("PBR-PET-2013). Our collective understanding of these topics has evolved significantly during the phase of data acquisition, and also after the publication of the original works I-III. Thus, the aims of the thesis, considering the evolving base of knowledge, are as follows:

- The aim of Study I was to assess the adaptation of [^{18}F]FMPEP-*d2* PET imaging in Turku PET centre from the standpoint of the results from existing validation studies. We further wanted to test for associations to demographic variables, such as whether there would be a sex difference in CB1R availability in humans. This was an essential step to reduce to risk of a type II error in a situation where the effect size of the change in a dependent variable was unknown. It is evident that unaccounted for confounders could considerably weaken the power of statistical tests, as well as complicating the interpretations of the results.
- The aim of Study II was to test for the presence of a central ECS dysfunction in first episode psychosis patients as compared to healthy controls. This was done simultaneously using two separate samples to reduce the risk of a type I error from a sampling bias. We also aimed to test whether CB1R availability would be associated with the use of antipsychotic medications, and the symptom domains of psychosis.
- Microglial and/or astrocyte dysfunction is one of the putative consequences of altered ECS signalling in psychotic disorders. Thus, the aim of Study III was to test for evidence of glial cell dysfunction in first episode psychosis as compared to healthy controls, measured with [^{11}C]PBR28 and PET, and since ECS signalling contributes to regulation

of microglial immune phenotypes, also whether central glial cell function would be associated to peripheral immune signalling in health or illness. We also examined for signs of altered peripheral cyto- and chemokine signalling in first episode psychosis as compared to population controls in a larger multi-site study. An attempt was made to replicate the previous result of increased CCL22 concentrations in a larger sample, and to characterize the result in a longitudinal setting. As in study II, this was done simultaneously using two separate samples to reduce the risk of a type I error from a sampling bias.

4 Materials and Methods

4.1 General methodology

4.1.1 Psychiatric phenotyping

Psychiatric disorders are defined as syndromes of clinically relevant symptoms occurring within discrete temporal patterns, and in the absence of a general medical or toxic causal factor. In psychiatric diagnostics, the clinical relevance of psychiatric symptoms and the presence of symptom syndromes are defined categorically. However, continuous definitions of psychiatric symptoms can be used to differentiate the phenotypic variation within the syndromes, and also in subclinical populations. The symptomatology of psychotic disorders clusters them into four symptom domains: positive, negative, cognitive and general symptoms. The severity of positive, negative and general symptoms can be assessed by grading reports of subjective experiences and observations of behaviour during structured clinical interviews. For instance, the interviewee is presented with a series of standardized probe question to elicit typical behavioural responses and subjective interpretations of intrapsychic phenomena. The responses are followed-up using interview techniques to ascertain the compatibility of subjective symptom concepts to clinical symptom concepts. Individual symptoms are then placed on an ordinal scale of increasing symptom severity defined by symptom form, content, duration, frequency, degree of conviction, and effect on behaviour or functioning. The cognitive symptom domain can be assessed by measuring test performance in standard cognitive tasks, such as tasks requiring working memory recall or continued attention. The difficulty level of the task can be increased gradually to reveal the individual limit of cognitive performance. Alternatively, task completion time or response quantity can be quantified, or response quality can be graded according to pre-existing rules.

4.1.2 Positron emission tomography

Positron emission tomography (PET) utilizes the fission of radioactive isotopes, which release positron particles when radioactive decay occurs. The positron

emitting isotopes, such as carbon-11 and fluoride-18 (^{11}C and ^{18}F respectively), are produced by bombarding a target material with charged particles accelerated in a cyclotron. A specific chemical bond within a specific tracer molecule is then substituted by a bond with the radioactive isotope, thus producing a positron emitting radiotracer. Radiotracer molecules are then administered into the blood circulation of the studied organism, whose biology determines the radiotracer's metabolism and elimination and also the kinetics of distribution of the radiotracer into tissue compartments. For instance, radiotracer binding to specific neurotransmitter receptors can be quantified using PET to study the alterations of neurotransmitter function in neurological and psychiatric disorders.

Radioactive decay occurs stochastically before and after radiotracer administration. The positron particles emitted by the radiotracer molecules are short lived as they are annihilated when colliding with the electrons of the molecules constituting the tissues. The annihilation of the antiparticles electron and positron produces two 0.511 MeV gamma-rays emitting in approximately opposite directions. A portion of these photons can be detected using a circular array of scintillators, which transform the energy of the gamma-ray into a registrable form using photo-multipliers or -diodes. Localization of the annihilation event is based on detecting the line of response of two temporally coincident events, and localizing the event to a segment of this line using image reconstruction techniques and the time evolved during the flight of the annihilation photon to reach the scintillator ring.

Coincidental annihilation events detected by the scintillator array during an emission scan session are recorded in one or more sinograms. A sinogram represents the angular array of lines of response plotted against its displacement from the centrum of the PET camera gantry. Thus, one line of a sinogram represents coincident events occurring on parallel lines of response, and the whole sinogram respectively represents all coincidental events within the 360° arc of the circular scintillator array. The sinogram can be reconstructed into a three-dimensional space using for instance ordered subset expectation maximization (OSEM), which iteratively updates the back-projected three-dimensional image matrix to maximize the correspondence of subsets of the forward-projected three-dimensional estimate and the sinogram.

The radiotracer is typically administered into the peripheral blood circulation via a venous cannula. The radiotracer distributes between the circulating blood fractions, non-specific tissues and specific binding targets as a function of time, and also as dictated by tracer characteristics, such as lipid solubility, affinity of specific binding, degree of non-specific binding, plasma protein binding, blood hemodynamics and peripheral metabolism of the tracer molecule into metabolites. The quantification of PET data is dependent on the properties of the used radiotracer and the studied tissue. Pertaining to this work, quantification can be done by modeling radiotracer distribution

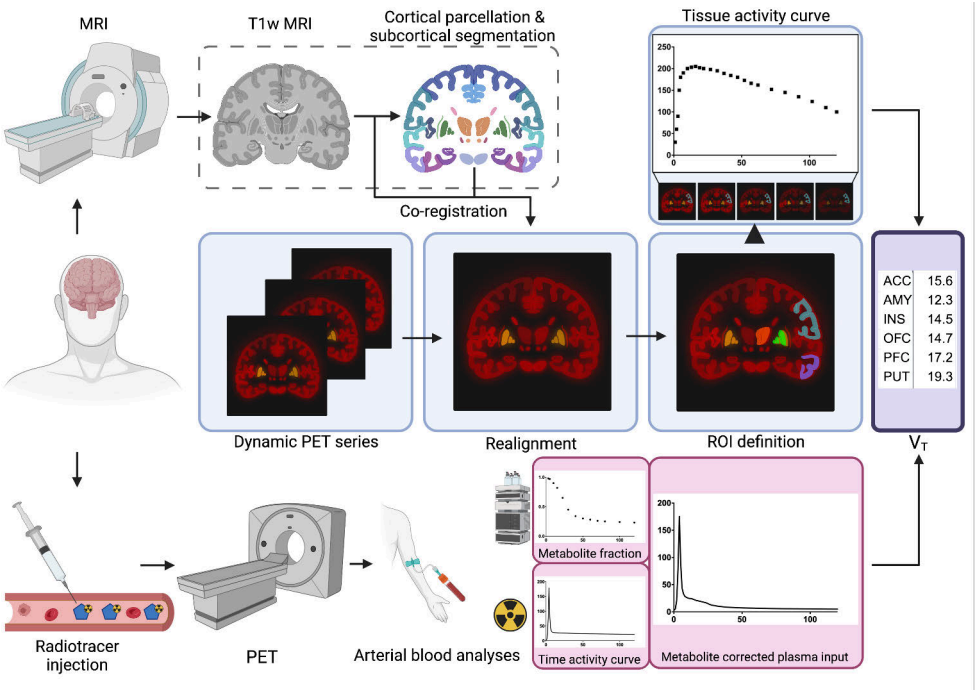
volumes (V_T) at the time of approximate kinetic equilibrium. V_T can also be defined as the proportion of specifically bound radiotracer activity concentration to activity concentration of unmetabolized radiotracer in peripheral blood at kinetic equilibrium. This proportion can be obtained using compartmental modeling, or models independent of compartmental assumptions such as multiple time graphical analysis or spectral analysis. Modeling of distribution volume can be done from tissue activity time-series of anatomically delineated volumes of interest, but also voxel-wise time series to produce parametric images of the distribution volume. **Figure 8**.

4.1.3 Magnetic resonance imaging

Studying the anatomy of the human body non-invasively in vivo is possible using magnetic resonance imaging (MRI). MRI scanners use static and gradient magnetic fields, and radiofrequency pulses, to respectively fix and displace the spin orientation of tissue ^{1}H-atom nuclei. This displacement, and subsequent relaxation to the static magnetic field, produces a radiofrequency signal, which is measured by a radiofrequency coil to produce information about tissue proton densities. Differences in tissue type proton densities allow differentiation of the different tissue classes using MRI, and thus provide anatomical information about the imaging target. For instance, cortical grey matter and white matter tissues can be differentiated by the differences in the time that the tissue proton magnetization vector takes to relax in parallel to the static magnetic field (T1-weighted MRI) after radiofrequency pulse excitation. Further, different tissue properties can be highlighted by measuring the time that the tissue proton magnetization vector takes to relax in a transverse orientation to the static magnetic field (T2-weighted MRI). While PET imaging theoretically provides only functional information about the human body, structural information can be integrated into the PET processing pipelines by probabilistic co-registration of structural MRI images into the PET time series. **Figure 8**.

4.1.4 Biofluid immunoassays

Proteins present in biofluids, such as peripheral blood serum, can be detected and quantified using a specific antibody capable of binding to the studied molecules. For example, the cytokine, chemokine or growth factor specific antibodies are bound to magnetized beads on a well plate. Individual biofluids are introduced into the wells in which the studied molecules simultaneously bind to their corresponding antibodies. Secondary antibodies are introduced, followed by wavelength specific fluorescent reporter molecules. The fluorescence signals, with specific wavelengths for each studied molecule, can be measured to quantify concentrations of the molecule to be assayed in the biofluid.



4.2 Study subjects

All study protocols were approved by the Joint Ethical Committee of the University of Turku and the Turku University Central Hospital. The studies were conducted according to the Declaration of Helsinki. Informed consent was obtained from all human subjects prior to their participating in the study and capacity of consent was assessed prior to obtaining consent.

4.2.1 Study I

Study I subjects were recruited as a part of the academic studies ”CB1-PET-2009” and the ”Neuroimaging platform for characterisation of metabolic co-morbidities in psychotic disorders” (METSY) -study. Healthy control subjects between 18–40 years of age were recruited via the national population registry, a local newspaper advertisement and an advertisement in local educational institutions. The somatic status of all the subjects was confirmed by medical examination, blood and urine tests, electrocardiography, MRI and their psychiatric status was evaluated using the structured clinical interview for DSM-IV axis I disorders (SCID-I). Lifetime substance use was documented, and current use was controlled with a urine screen prior to the PET scan for brain CB1-receptor availability. Exclusion criteria were: 1) lifetime Axis I -disorder, 2) lifetime medical disorder affecting the brain, 3) illicit

◄**Figure 8.** General methodology of PET imaging using [^{18}F]FMPEP-*d2*, [^{11}C]MEPPEP and [^{11}C]PBR28. The brain of the subject is first scanned with MRI to obtain T1-weighted (T1w) images to use for structural reference of the PET data. This structural data is then refined into spatially categorical area definitions using a probabilistic atlas, or automated cortical parcellation and cortical segmentation as depicted in the figure. Second, the patient undergoes a dynamic PET scan, which can be from 60min to 120 min in length. At the start of the PET scan, a positron emitting tracer, which has been labeled with a radioactive isotope such as [^{11}C] or [^{18}F], is injected into the subject's venous bloodstream. The distribution and kinetics of the radiotracer are then measured along a series of time frames using the PET camera. This results in a dynamic series of PET images. A series of arterial blood samples are obtained simultaneously to the PET scan to measure the kinetics of the radiotracer in blood. Since tracer metabolites can contain the radioactive isotope after metabolism of the radiotracer, these are measured using methods such as high-pressure liquid chromatography (HPLC) or thin layer chromatography (TLC), resulting in a time series of the fraction of unmetabolized tracer. The measured time-series of radioactivity in plasma, which is separated from centrifuged whole blood samples, is then corrected for the fraction of radioactivity arising from radioactive metabolites. The obtained metabolite corrected plasma radioactivity time-series represents the parent input of the tracer into further tissue compartments. This is what drives the kinetics of the tracer between the blood-brain interface, and the brain-molecule interface. After the PET scan, the singular time frames of the dynamic PET series are realigned to a reference frame to correct for movement of the head between the frames. The T1w MRI is then co-registered to the sum of the re-aligned PET image series using affine transformations. These transformations are also applied to the categorical area definitions, which can be then used to delineate the tissue radioactivity time series from specific anatomical areas, such as depicted here using anatomical masks (orange, green, purple and blue) superimposed on the stack of PET images. The tracer activity from these areas can then be measured from each PET frame to form a tissue activity curve. The distribution and the kinetics of redistribution of the tracer can be then mathematically modelled by using the metabolite corrected plasma time activity curve as the parent input, and the PET tissue time activity curve as the non-specific (NS) and specific (S) tracer binding compartments. The kinetic properties of the obtained model can be used to approximate for example the fraction of NS+S/parent binding at kinetic equilibrium, or in other words, the distribution volume (V_T) of the tracer in selected regions of interest (ROI), such as the anterior cingulum (ACC), amygdala (AMY), etc.

substance use within 2 months prior to PET scanning, 4) pregnancy, 5) over 4mm frame-to-frame movement during the PET scan. Altogether 11 male and 11 female healthy volunteers were included in the statistical analyses to examine sex differences in [^{18}F]FMPEP-d2 V_T.

4.2.2 Study II

Study II consisted of two independent samples of male first episode psychosis patients and age matched healthy control subjects recruited as a part of the "Neuroimaging platform for characterisation of metabolic co-morbidities in psychotic disorders" (METSY) -study. The first episode psychosis patients in the Turku study were primarily using antipsychotic medication, but were of more recent onset compared to the unmedicated patient sample in the London study.

For the Turku study, seven male first-episode psychosis patients between 18-40 years of age were recruited from psychiatric inpatient wards and outpatient clinics of Turku health services and the Hospital Districts of Southwest Finland and Satakunta. In the Turku study, first-episode psychosis was defined as the presence of DSM-IV Axis I psychosis diagnosis with the onset of treatment for first episode of symptoms within two years. The date of onset of prodromal and psychosis symptoms, and the presence of any previous psychotic episodes, were further assessed using the structured interview for prodromal syndromes (SIPS 5.0). Lifetime substance use was documented, and current use was controlled with a urine screen prior to the PET scan for brain CB1-receptor availability. Altogether 11 male healthy volunteers and 7 male first episode psychosis patients were included in the statistical analyses for group differences in [^{18}F]FMPEP-d2 V_T. In Turku, the male healthy control subjects participating in study II were recruited as described above for study I.

In the London study, 20 male first-episode psychosis patients between 18-60 years of age with diagnoses of schizophrenia or schizoaffective disorder were recruited from psychiatric health services in London, United Kingdom. Patient subjects had been free of antipsychotic medication for 6 months.

Exclusion criteria for all study subjects were: lifetime substance dependency diagnosis, illicit substance use within 1 month prior to PET scanning, positive results for cannabis or other substances on urine toxicology screening, chronic medical or neurological condition affecting the brain, history of head trauma with loss of consciousness, neurodevelopmental disorders, over 5mm frame-to-frame movement during the PET scan. Additionally, healthy controls had no history of lifetime Axis I disorders as defined by SCID-I non-patient edition (Turku) or SCID-I patient edition (London).

4.2.3 Study III

Study III consisted of two independent cohorts of male and female first episode psychosis patients (n=129) recruited from the psychiatric clinics of the City of Helsinki and the Helsinki University Hospital in the Helsinki Early Psychosis Study (HEPS), and in the Turku Early Psychosis Study (TEPS), from the clinics of the City of Turku and the Hospital district of Southwest Finland. All primary psychotic disorders were included, whereas substance-induced psychoses and psychotic disorders due to a general medical condition were excluded. Controls (n=130), matched by age, sex, and place of residence, were recruited through the Population Register Centre. All participants were 18–40 years of age. Psychotic disorders and chronic neurological or endocrinological diseases were exclusion criteria for the controls.

A subgroup of first-episode patients (n=14) and healthy controls (HC) (n=15) were recruited from the TEPS study for the [^{11}C]PBR28 PET study. The somatic status of all the PET subjects was confirmed by medical examination, blood and urine tests, electrocardiography, SCID-I, and the Structured interview for prodromal syndromes (SIPS version 5.0). Pregnancy was ruled out by urine and/or blood screening. Subjects with a chronic medical or neurological condition affecting the brain, history of head trauma with loss of consciousness, and neurodevelopmental disorders were excluded. All PET subjects refrained from substance use two months prior to PET and substance use was screened using a urine toxicology test. Subjects with a lifetime substance dependency diagnosis, or who had used any illicit substances two months prior to scanning, were excluded. Healthy controls with a history of DSM-IV Axis I diagnosis were excluded from the PET study.

4.3 Analyses of peripheral blood cytokines, chemokines and growth factors

Fasting blood samples were collected at 8–10 a.m. Serum samples coagulated at room temperature (max. 2 hours) and were then centrifuged, aliquoted and stored at -80°C. In the PET study, fasting serum was sampled before radiotracer injection at 8–9 a.m. on the day of the PET procedure.

The serum concentrations of cytokines, chemokines and growth factors were analyzed using the 38-plexed Milliplex MAP Kit (cat.no. HCYTMAG-60K-PX38) according to the manufacturer's recommendations (Merck-Millipore Corp., Billerica, MA, USA). Additionally, CCL22 concentrations were reanalyzed using unthawed serum samples from the subjects who participated in [^{11}C]PBR28 PET. This data was complemented with analyses of serum CCL17 levels based on the association of elevated CCR4 receptor ligand CCL22 with FEP in the complete study sample. Reanalyses of CCL22 were done using the same 38-plexed Milliplex plate to avoid a methodological bias, while CCL17 levels were analyzed in duplicate reactions using a 1:2 dilution of the serum samples with the commercial Human CCL17 ELISA kit according to the manufacturer's instructions (Thermo Scientific, Waltham, Massachusetts, USA).

4.4 [^{18}F]FMPEP-*d2* and [^{11}C]MEPPEP positron emission tomography

The specific CB1R radiotracer [^{18}F]FMPEP-d2 was used to quantify CB1R availability in Study I and Study II, while [^{11}C]MEPPEP was used in Study II to quantify CB1R availability in the study subjects recruited in London. Detailed

methods of radiotracer synthesis, positron emission tomography, and radiotracer blood activity measurements, as well as metabolite analyses can be found in the methods section and method supplements of Studies I and II. The availability of CB1R, in regions of interest or voxel-wise, was quantified using Logan graphical analysis. A radiotracer plasma activity time series, corrected for radiometabolites, was used as the input function for the model. The decay corrected positron emission time series represented the tissue activity concentration in the model. In Study I, regions of interest were derived by applying the Freesurfer analysis suite to the individual T1 weighted structural MRI and co-registering the individual anatomical information to the motion corrected PET time series. In Study II, the Hammersmith atlas was used for anatomical definition of the PET time series.

4.5 [^{11}C]PBR28 positron emission tomography

The specific translocator protein radiotracer [^{11}C]PBR28 was used to quantify TSPO availability in Study III. Detailed methods of radiotracer synthesis, positron emission tomography, and radiotracer blood activity measurements, as well as metabolite analyses can be found in the methods section and method supplements of Study III. The availability of TSPO in regions of interest was quantified using a 2-tissue compartment kinetic model. Radiotracer plasma activity time series, corrected for radiometabolites, was used as the input function for the model. The decay corrected positron emission time series represented tissue activity concentration in the model. Blood volume was fixed at 5%. In the voxel-wise analyses, the availability of TSPO was quantified using Logan graphical analysis. Regions of interest were derived by applying the Freesurfer analysis suite to the individual T1 weighted structural MRI and co-registering the individual anatomical information to the motion corrected PET time series.

4.6 Statistical methods

The statistical tests used in Studies I–III are presented in **Table 5.**

Table 5. Statistical methods used in studies I-III

Statistical method	**Study I:**	**Study II**	**Study III:** Peripheral immune function	**Study III:** TSPO PET imaging
Q-Q plot	X	X		X
Shapiro-Wilks test	X	X		X
Mauchly test for sphericity	X	X		X
Student's t-test	X	X		X
Mann-Whitney U-test	X	X		X
Chi-squared test	X	X	X	X
Pearson's r correlation	X	X	X	X
Repeated measures ANOVA	X	X		X
Greenhouse-Geisser correction	X	X		X
Bonferroni correction		X	X	
FWER-correction	X			
FDR-correction	X			
Wilcoxon signed-rank test			X	
Wilcoxon two-sample test			X	
Spearman's rank correlation			X	
General linear model			X	

4.7 Ethical considerations

The study protocols were approved by: 1. the joint ethics committee of the University of Turku and the Turku University Central Hospital for studies I–III conducted in Turku, 2. King's College London ethics committee for the part of study II conducted in London, and 3. the ethics committee of the Hospital District of Helsinki and Uusimaa for the part of study III conducted in Helsinki. All studies were conducted according to the Declaration of Helsinki. Capacity for consent was assessed and informed consent was obtained from all human subjects prior to their participating in the study.

5 Results

5.1 Study I: Sex difference in brain CB1 receptor availability in man

The V_T of [^{18}F]FMPEP-*d2* was higher in healthy males compared to females ($F(1)=13.150$, $p=0.002$) with a group by region interaction ($F(2.528)=7.114$, $p=0.001$). The availability of CB1R was higher in males compared to females ($p<0.05$) in all brain regions, **Figure 9**. The largest effects were present in the occipital cortex, the parietal cortex and the posterior cingulate cortex. A voxel-wise t-test showed one large significant cluster of higher [^{18}F]FMPEP-*d2* V_T in males compared to females ($kE\geq250679$, $p_{FDR\text{-}corr}<0.001$), **Figure 10**.

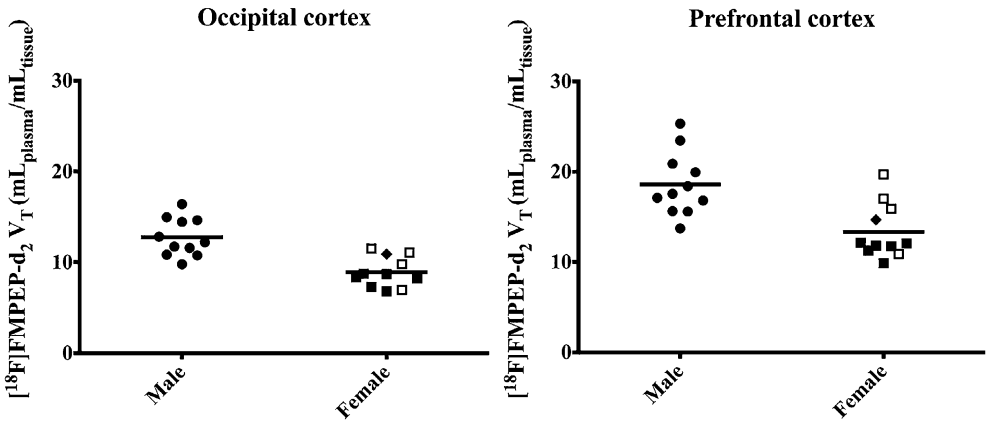


Figure 9. [^{18}F]FMPEP-*d2* V_T in the occipital cortex and prefrontal cortex of healthy males and females separately. Males show higher CB1R availability than females with regional differences in effect sizes. Females using estrogen containing combined oral contraceptives are marked as closed squares (■). One subject was in menopause (◆). The pattern of variance suggests that the hormonal status of female subjects might affect the availability of CB1R. Reprinted from Laurikainen et al. (2019) under the terms of the C-C BY-NC-ND license.

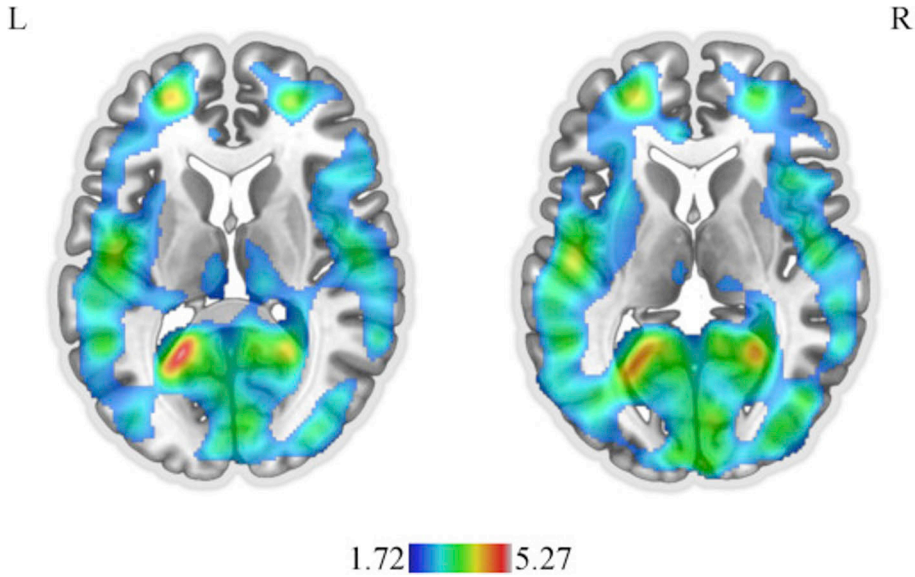


Figure 10. Parametric comparison of [^{18}F]FMPEP-*d2* V_T shows one statistically significant cluster of difference between healthy males (n=11) and females (n=11). The color bar denotes different values of T, which remained significant after correction for multiple comparisons ($p_{FDR\text{-}corr}$<0.001, kE ≥ 250679). T-value of T=1.72 denotes the height threshold, while T=5.72 was the maximum peak value at the coordinates: x=−21 mm, y=−54 mm, z=14 mm. L: left,; R: right. Reprinted from Laurikainen et al. (2019) under the terms of C-C BY-NC-ND license.

5.2 Study II: In Vivo availability of Cannabinoid 1 Receptor Levels in Patients With First-Episode Psychosis

The V_T of the two CB1R radiotracers, [^{18}F]FMPEP-*d2* and [^{11}C]MEPPEP, were lower in FEP groups of 7 and 20 subjects compared to HC groups of 11 and 20 subjects respectively. In the Turku study, the V_T of [^{18}F]FMPEP-*d2* was lower in the anterior cingulate cortex (ACC) (Hedges' g=1.2), hippocampus (Hedges' g=1.4), striatum (Hedges' g=1.9), and thalamus (Hedges' g=1.4). In the London based study, the V_T of [^{11}C]MEPPEP was lower in the ACC (Hedges' g=0.8), hippocampus (Hedges' g=0.5), striatum (Hedges' g=0.4), and thalamus (Hedges' g=0.7). **Figure 11**. Anterior cingulate cortex [^{11}C]MEPPEP V_T was directly associated with cognitive functioning (r=0.60; p=0.01) while hippocampal [^{11}C]MEPPEP V_T was inversely associated with PANSS total symptom scores (r=-0.50; p=0.02) in unmedicated FEP patients.

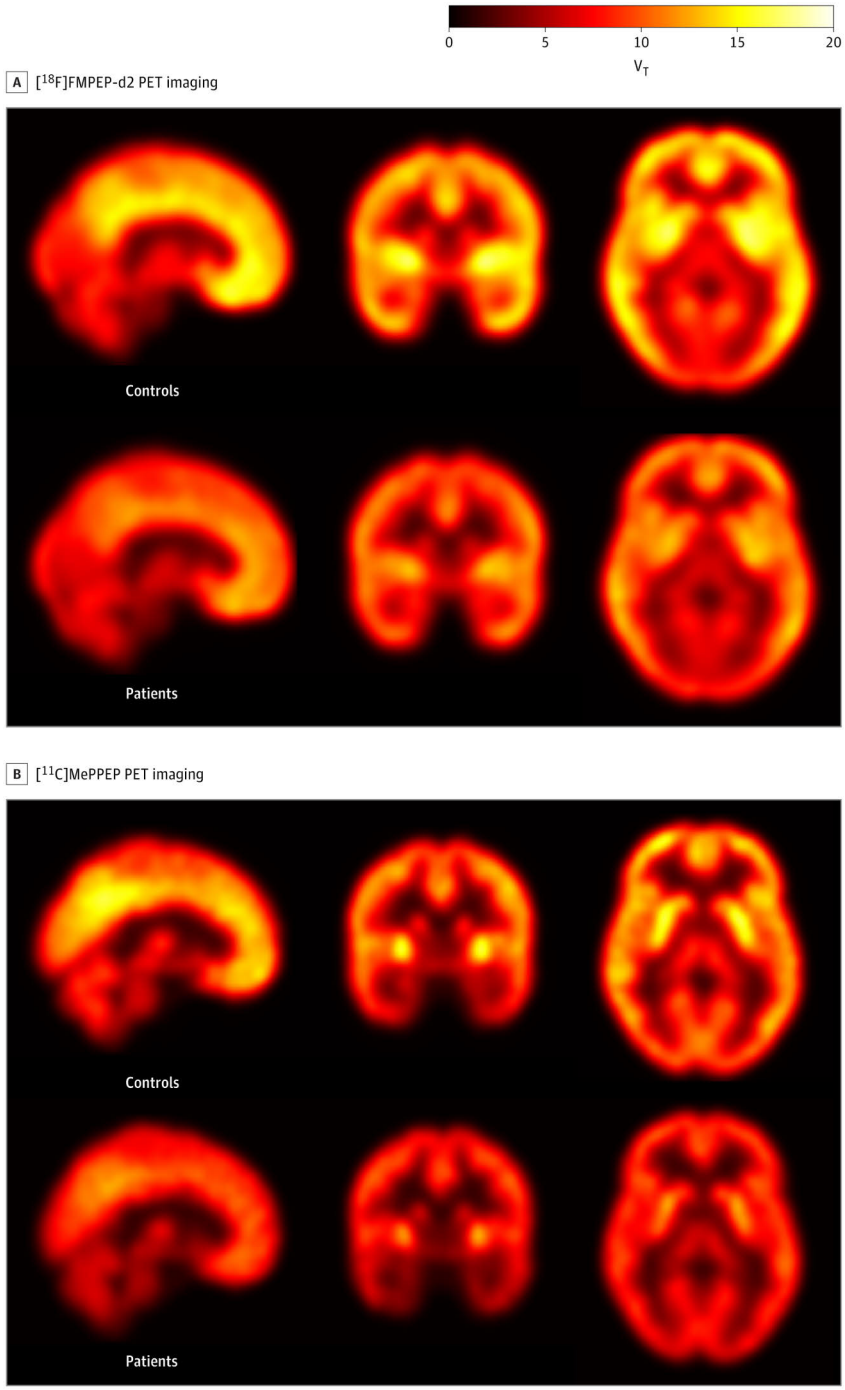


Figure 11. Differences in CB1R availability (V_T) between medicated (A) and antipsychotic free (B) psychosis patients relative to healthy individuals. Reprinted from Borgan et al. (2019) under the terms of the C-C BY license.

5.3 Study III: Elevated serum chemokine CCL22 levels in first-episode psychosis: associations with symptoms, peripheral immune state and in vivo brain glial cell function

Serum concentrations of C-C motif chemokine 22 (CCL22) were significantly higher in FEP patients compared to controls ($p<0.0001$; Cohen's $d=0.696$), and the difference remained statistically significant after Bonferroni correction for multiple testing of 38 cyto- and chemokines. CCL22 levels were higher in both the HEPS ($p<0.0001$) and the TEPS ($p<0.0001$) samples separately. In a general linear model (adjusted $R^2=0.22$) FEP status was a significant factor of elevated CCL22 ($\beta=245.3$, $p=0.0019$), while smoking ($\beta=308.0$, $p=0.0004$), olanzapine use ($\beta=209.7$, $p=0.036$) and obesity ($\beta=303.2$, $p=0.0042$) were also associated with concentrations of CCL22. Levels of CCL22 had numerically more significant correlations with other cyto- and chemokines in the controls in comparison to FEP patients. Cytokines related to adaptive immune system functions were the most important signalling nodes in controls, while cytokines and growth factors related to innate immune functions were more prominent in FEP. High and low CCL22 groups were defined based on the median FEP CCL22 concentration (1277.5 pg/mL) to further explore the functional significance of elevated CCL22 levels. The concentrations of several cytokines, chemokines and growth factors, such as CX3CL1, were higher in high CCL22 FEP patients compared to high CCL22 controls or low CCL22 FEP patients. This suggests that there may be a more extensive dysregulation of the immune system in the context of high CCL22 levels. Further, CCL22 levels did not change significantly in either FEP or control subjects between the follow-up measurements. CCL22 was also persistently elevated at the one-year follow-up in FEP patients of the high CCL22 group as compared to low CCL22 FEP patients.

The V_T of [^{11}C]PBR28, covaried for the effect of TSPO rs6971 genotype, was lower in FEP patients in comparison to HCs ($F(1,25)=5.635$, $p=.026$; Cohen's $d=0.936$) without a group by region interaction ($F(3.486)=1.293$, $p=0.281$). **Figure 12** This effect remained statistically significant when including BMI ($F(1,24)=7.003$, $p=0.014$), or BMI, age and sex ($F(1,21)=4.655$, $p=0.043$) as covariates in the model. Leaving out any one subject from the analysis of [^{11}C]PBR28 V_T group effects, covaried for the TSPO rs6971 genotype, did not alter the significance of the result. Linear regression models were estimated to assess the relationships between whole brain grey matter [^{11}C]PBR28 V_T and CCL17 or CCL22 serum concentrations in a combined sample including both FEP and HC subjects. TSPO availability was significantly associated to levels of CCL17 ($F(1,26)=4.361$, $p=0.047$, $R^2=0.144$). For CCL22, a trend level association to TSPO availability was found ($F(1,26)=3.017$, $p=0.09$, $R^2=0.104$). Serum levels of CCL17 were moderately correlated to levels of CCL22 ($\rho=0.498$, $p=0.008$).

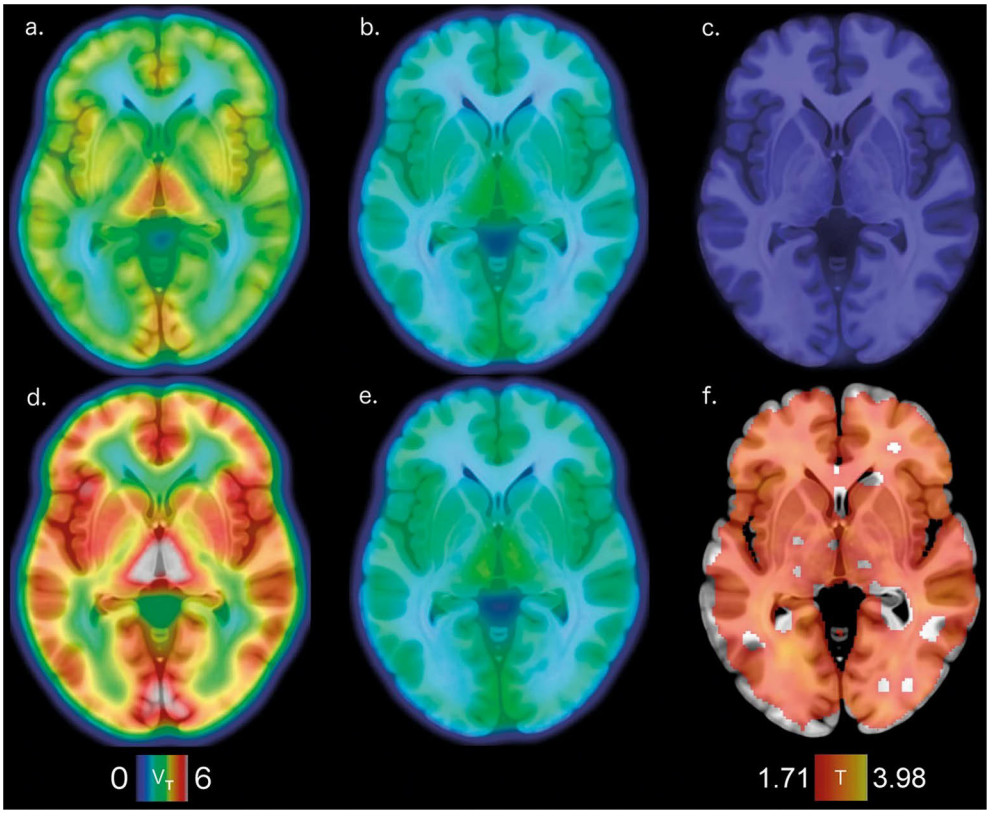


Figure 12. Differences in TSPO availability (V_T) between: **a.** high-affinity binder (HAB) first-episode psychosis patients (FEP), **b.** medium-affinity binder (MAB) FEPs, **d.** HAB healthy controls (HC), and **e.** MAB HCs. The V_T of one low-affinity binder FEP subject is shown in **c.** to demonstrate the level of regional non-specific binding in the FEP population. A statistical parametric mapping analysis shows one cluster of lower V_T in FEPs compared to HCs. The left color bar denotes the V_T values represented by different colors in **a.**–**e.** The right color bar denotes the T-values represented by the red-to-yellow gradient in **f.** Reprinted from Laurikainen et al. (2020) under the terms of the C-C Attribution 4.0 license (http://creativecommons.org/licenses/by/4.0/).

6 Discussion and Conclusions

6.1 Methodological considerations of measuring CB1R availability with positron emission tomography

The application of [^{18}F]FMPEP-*d2* PET imaging at the Turku PET centre seemed adequate for use in clinical studies. The radiotracer was synthesized with high radiochemical purity (95%) and very high molar radioactivity (>500 GBq/μmol). These translated to an injected mass of <194 ng or <0.41 nmol in Study I, which would correspond to <<1% occupancy when applying the same assumptions as used by Terry, Hirvonen, Liow, Zoghbi, et al. (2010). Consequently, endogenous ligands would not be displaced in significant amounts due to low radiotracer occupancy. Both Logan plot and 2-tissue compartmental modelling of 120min of PET data, using a metabolite corrected arterial input, produced highly correlated values of V_T. The magnitude of V_T was comparable to the results obtained from previous [^{18}F]FMPEP-*d2* studies of healthy humans. (Hirvonen et al., 2012; Hirvonen et al., 2013; Terry, Hirvonen, Liow, Zoghbi, et al., 2010) We did not measure the [^{18}F]FMPEP-*d2* plasma free fraction in Study I or II, since previous attempts indicated that the test-retest variability was as high as ∼50%, and that it would introduce significant noise to the data. (Terry, Hirvonen, Liow, Zoghbi, et al., 2010) Correcting for the plasma free fraction would have demanded substantially larger study samples, which was not feasible given the restricted clinical population in Study II. However, plasma free fractions were measured for the [^{11}C]MEPPEP PET in Study II, and the difference of plasma free fraction in healthy individuals as compared to first episode psychosis patients was not significant (HC: 0.19, FEP: 0.16; p=0.09). There were no differences in plasma free fraction in other studies using healthy controls and patient samples, (Hirvonen et al., 2012; Hirvonen et al., 2013) but this cannot be generalized to apply to studies using samples of FEP patients. Thus, we cannot exclude the possibility that differences in plasma free fraction contributed to the effect size of the result of [^{18}F]FMPEP-*d2* measurements in Study II.

6.2 Confounders of measuring CB1R availability

Study I showed that healthy females have a lower CB1R availability than males. However, the hormonal status of the female subjects was not controlled for, and the female sample contained subjects using hormonally active contraceptives. Although an ECS sex difference seems probable in the light of the plethora of cumulated evidence reviewed previously, these confounders probably contributed to the high effect size of sex differences in Study I. The distribution of female CB1R V_T, stratified according to the use of hormonal contraceptives, supports this proposal (**Figure 9**). Previously, Hirvonen et al. (2012) and Tuisku et al. (2019) reported associations between CB1R availability and body mass index (BMI), age, or ethnicity. Van Laere et al. (2008) showed that the association with age might be different in males and females. Age, BMI, or other demographic variables did not associate to CB1R V_T in Studies I or II but these were probably underpowered to detect associations with age, BMI or past cannabis exposure given that the distributions of these variables were restricted by design.

6.3 Endocannabinoid system dysregulation in first episode psychosis

In Study II, the availability of anterior cingulate cortex, hippocampus, striatum and thalamus CB1R was lower in male FEPs compared to controls. The [^{11}C]OMAR PET study of CB1R availability in chronic schizophrenia by Ranganathan et al. (2016) is consistent with the results obtained in Study I. The combined available data suggests that the dysregulation of the ECS was not related to medication status or duration of illness. Comparison with the other available PET studies conducted by Wong et al. (2010) and Ceccarini et al. (2013) is methodologically problematic. Wong et al. (2010) reported a singular significant finding of elevated CB1R availability in the pons. This raises questions about the nature of the measured signal, since the pons is relatively devoid of CB1R and shows relatively high degrees of non-specific binding. The study by Ceccarini et al. (2013) on the other had a respectable sample size of 67 schizophrenia patients and 12 controls, but modified standardized uptake ratios were used as the primary endpoint. This renders the result incompatible with studies using kinetic modelling of V_T using metabolite corrected plasma input. Notably, the result of higher CB1R mSUV in chronic schizophrenia resembled those obtained from ex vivo autoradiography studies.

Excluding methodological confounders, a group difference of CB1R V_T can generally be due to group differences in: 1) receptor protein density, 2) radiotracer displacement by endogenous ligands, 3) radiotracer affinity to CB1R, 4) non-displaceable binding, or 5) receptor protein distribution across intra- and extracellular compartments.

6.4 The molecular biology behind CB1R availability

Neither [^{11}C]MEPPEP nor [^{18}F]FMPEP-*d2* are displaced by the AEA analogue methanandamide. (Terry, Hirvonen, Liow, Zoghbi, et al., 2010) Displacement by AEA or 2-AG is likewise improbable due to their affinities for human CB1R (AEA: K_i=239.2nM; 2-AG: K_i=3423.6nM), relative to those of [^{11}C]MEPPEP (K_i=9.6nM) and [^{18}F]FMPEP-*d2* (K_i=9.6 nM). (McPartland et al., 2009; Terry, Hirvonen, Liow, Zoghbi, et al., 2010) However, CB1Rs can exist in two ligand-bound conformations. (Hua et al., 2017; Shao et al., 2016) Both agonist and antagonist bound conformations affect the shape of the ligand binding pocket. (Manandhar et al., 2022) Antagonists and inverse agonists, such as [^{11}C]MEPPEP and [^{18}F]FMPEP-*d2*, seem to have stronger molecular interactions with specific binding pocket amino acids of the inactive conformation of CB1R, while agonists, such as AEA and 2-AG, prefer different molecular interactions within the binding pocket in the active conformation. (Manandhar et al., 2022) Both ortho- and allosteric modulators have the potential to shift the conformation of CB1R. An increased AEA tonus in FEP patients, as suggested by prior studies, (Garani et al., 2021; Minichino et al., 2019) could potentially shift the receptor pool towards the active conformation. This would in turn reduce the binding affinity of inverse agonist radiotracers to the CB1R. The lower CB1R V_T in psychosis patients as compared to controls could thus be due to changes in the ligands' affinity for the receptor, since endogenous ligands or the radiotracers used do not displace each other, and V_T is proportional to available receptor density ($B_{available}$) as well as receptor affinity (K_D).

CB1R binding of selected endocannabinoid agonists promote the internalization of the CB1R molecule. (Daigle et al., 2008) It has not been proven whether the binding of inverse agonist radioligands represents this population of internalized receptors. Differences in affinity, lipophilicity, and tracer kinetics could affect the proportion of compartments represented by different PET radiotracers. However, the continuing signalling activity, and association to second messenger proteins, of internalized receptors indicates that this receptor population is in the active conformation. (Nogueras-Ortiz & Yudowski, 2016) Thus, the majority of [^{11}C]OMAR, [^{11}C]MEPPEP and [^{18}F]FMPEP-*d2* binding most likely is attributable to the CB1Rs present on neuronal cell membranes regardless of putative radioligand binding to internalized CB1Rs, since antagonists and inverse agonists favour the inactive CB1R conformation. Further, Lee et al. (2015) showed that the majority of CB1Rs reside on the surface of hippocampal GABA interneurons, and that increased levels of 2-AG or AEA do not seem to affect the proportion of membrane-bound/internalized receptors significantly in vitro. Assuming that the result by Lee et al. (2015) is generalizable to non-hippocampal neurons in vivo, and that the results by Manandhar et al. (2022) translate to variable tracer affinities depending on receptor conformation, then binding of inverse antagonist radiotracers should be a

product of conformation changes due to ortho- or allosteric ligands, and the density of membrane-bound receptors.

6.5 Comparative discussion of ECS dysregulation in psychotic disorders

The available ex vivo autoradiography studies suggest that CB1R density is rather unchanged or increased, not decreased (**Table 2**). The largest autoradiography studies reported by Volk et al. (2014) and Jenko et al. (2012), using tissue sections and tissue homogenate respectively, both show increased CB1R densities in chronic schizophrenics. Out of the autoradiography studies reviewed in **Table 2**, The experiment conducted by Volk et al. (2014) was the only one using tissue homogenate samples. Similar results from these autoradiography studies, using tissue sections or homogenates, suggest that the reported increased densities represent both the membrane-bound, as well as the intracellular receptor populations. It has been reported that increased CB1R protein expression can be due to AEA induced activation of the intracellular Akt-pathway. (Laprairie et al., 2014) Immunohistochemistry studies of CB1R immunoreactivity however suggest that less CB1R proteins are available for detection by antibodies in ex vivo tissue sections (**Table 2**). The study by Volk et al. (2014) also demonstrated that while ex vivo tissue sections of schizophrenia patients are less immunoreactive to CB1R antibodies, and show less Cnr1 mRNA expression, they reciprocally bind more of the inverse agonist [^{11}H]OMAR. Post-mortem effects and handling of ex vivo tissues most likely lead to variable degrees of disintegration of tissue compartment boundaries, while the release of neurotransmitters during the agony of death, and relaxation of ion gradients and membrane potentials have unknown consequences for the state of the ECS. In addition, a post-mortem change in ECS dynamics, either related to chronic illness or death, is possible given lower AEA and higher 2-AG concentrations in tissue homogenates of the cerebellum, hippocampus and prefrontal cortex of schizophrenia patients in comparison to controls. (Muguruza et al., 2013) It is known that CSF and blood AEA concentrations are elevated in patients with early as well as chronic psychoses, (Garani et al., 2021; Minichino et al., 2019; Potvin et al., 2020), but unfortunately in vivo brain tissue concentrations of AEA and 2-AG in non-chronic schizophrenia have not been measured for comparison. While the availability of FAAH, the primary metabolizing enzyme of AEA, seems to be unchanged in the brain of psychosis patients as compared to healthy controls, both brain levels of FAAH and AEA do seem to be inversely associated to symptom severity. (Garani et al., 2021; Watts et al., 2022; Watts et al., 2020)

The bidirectional changes in post-mortem CB1R immunohistochemistry and mRNA measures compared to autoradiography measures in schizophrenia can be

due to three reasons; 1) post-mortem changes affecting the affinity of radiotracers to CB1R, 2) post-mortem changes in the tissue compartments available for radiotracer/antibody binding, or 3) fundamental methodological differences leading to different aspects of the ECS being measured by radiotracers and immunohistochemistry. The Anti-CB1R-L15-antibody used by Eggan et al. (2008), Eggan et al. (2010) and Volk et al. (2014) has been shown to be exclusively specific for CB1R on inhibitory neurons. (Eggan & Lewis, 2007) The reciprocal post-mortem result of higher CB1R radiotracer binding and lower immunoreactivity could thus be explained by differences in radiotracer and antibody sensitivity or selectivity. Furthermore, a sampling bias in the immunohistochemistry studies cannot be excluded, since the majority of the immunohistochemistry studies were done with partially overlapping samples, while the autoradiography studies used discrete samples (**Table 2**). Notably, an unrelated sample from an immunohistochemistry study performed by Koethe et al. (2007) did not find significant differences of CB1R immunoreactivity between schizophrenia patients and their controls. The singular immunoblotting study, which is not affiliated to the above-mentioned overlapping samples, detected decreased CB1R protein expression in 11 medicated schizophrenia patient in comparison to controls, while 11 non-medicated patients did not significantly differ from their controls. Ultimately, the differences in sensitivity/selectivity of CB1R immunohistochemistry compared to autoradiography hampers attempts to compare these results directly. Peripheral measures of CB1R are a poor substitute for indexing central CB1R functioning, but nonetheless, peripheral blood mononuclear cells express more CB1R in psychosis patients than controls. (Minichino et al., 2019)

6.6 The biological implications of ECS dysregulation

It is plausible to suggest that the effects of increased AEA synthesis on the conformation of membrane-bound CB1R contributes to decreased CB1R availability, when measured in vivo with inverse agonist PET ligands. At the same time, the CB1R protein concentrations could be counterintuitively increased secondary to the effects of AEA on Cnr1 gene expression. We do not know whether mechanisms unrelated to AEA and 2-AG, such as regulation of receptor trafficking by CRIP1a, could lead to increased receptor internalization in psychotic disorders. If so, the results obtained from post-mortem CB1R autoradiography studies might better reflect the whole receptor population, possibly without the in vivo effects of increased AEA signalling on receptor conformation. It can be speculated that increased receptor trafficking of internalized receptors could also explain a part of the rapid normalization of CB1R availability after cessation of THC exposure.

(D'Souza et al., 2016) It is not known whether the increases in AEA are sufficient to explain the effect sizes of the difference in CB1R availability. Allosteric modulators of CB1R and receptor heteromerization could also contribute to the effect.

Altogether, the cumulated evidence base is consistent with the following hypotheses: 1) the functioning of central ECS signalling is altered in psychosis due to increased AEA tonus, 2) receptor conformation and ligand binding are shifted towards the active conformation in psychosis, and 3) the distribution of CB1Rs between membrane-bound and intracellular compartments is affected by psychosis. The presence of altered 2-AG signalling, the putative functional consequences of changes in receptor conformations and distribution, and the direction of changes in the amount of total CB1R protein are still unclear. It is evident that more studies will be needed to clarify the relationship of ECS dysregulation to the clinical symptomology as well as delineating the underlying intermediating mechanisms.

AEA CSF concentrations seem to be inversely associated to symptom severity in psychosis, and to reduced psychotic transition in genetic and clinical high-risk populations. (Garani et al., 2021; Koethe et al., 2009; Koethe et al., 2019; Minichino et al., 2019) It has been claimed that brain FAAH availability is also inversely related to the positive symptoms of psychosis, but also to longer durations of illness and untreated psychosis. (Watts et al., 2020) Additionally, total PANSS symptom scores were inversely, and cognitive performance in the digit symbol substitution test were directly associated to CB1R availability in Study II. The direct associations of CSF AEA to symptoms of depression in mood disorders however complicate the interpretation of association to the total PANSS sum scores, (Garani et al., 2021) since there is a considerable phenomenological overlap between psychotic and mood disorders. Furthermore, total PANSS scores were directly associated with CB1R availability when measured with [^{11}C]OMAR. (Ranganathan et al., 2016) These problems of interpreting discrepant associations of CB1R availability with psychosis symptom severity, and the putative confounding effect of depression restrict interpretations about the functional role of increased AEA tonus in psychotic disorders.

The integral links between the ECS, the HPA-axis and the hypothalamic-pituitary-gonadal hormone systems also suggest that ECS dysregulation might be related to the hormonal disturbances evident in psychotic disorders. Unfortunately, the association of striatal and hippocampal CB1R availability to anterior cingulate cortex glutamate levels, measured by PET and MRS, (Borgan et al., 2021) does not help in deciphering this issue since the result has implications for both anterior cingulate cortical regulation of amygdalar control over hypothalamic hormonal functions, as well as an involvement in the regulation of cortical E/I-balance. An increased AEA tone in CCK+ GABA interneurons is also able to contribute to mesolimbic dopamine dysregulation. AEA seems to inhibit the depolarization-

induced inhibition transmitted by activity-dependent retrograde 2-AG signalling. (Lee et al., 2015; Maccarrone et al., 2008) For example, disinhibited CCK+ interneurons could inhibit PV+ GABA interneurons, which could in turn lead to disinhibition of pyramidal neuron projections to the midbrain. However, the network of inhibitory interneurons is complex, and CCK+ interneurons also directly inhibit glutamatergic cells. The increased AEA tonus in these cells could also be due to a compensatory effort secondary to some other pathology. The inverse associations between AEA and the severity of the symptoms of psychosis would be in line with this concept. The endocannabinoid system can also be considered to act as part of a cortical filter, which modulates the topography of cortical representations and therefore a dysregulated ECS could present clinically as a loosening of associations and disorganized thought processes. Both loosening of associations, as well as increased levels of [^{18}F]DOPA, have been documented in THC intoxication states. Cortical representations could also be affected by the influence of the ECS on the development of cortical column connectivity, microglia-mediated synaptic plasticity, as well as through the actions of the ECS on astrocyte-mediated short-term modulation of synaptic activity and homeostasis. The clinical relevance of ECS dysregulation in psychosis has been demonstrated by early observations of the therapeutic potential of cannabidiol (CBD) treatment in psychotic disorders. (Leweke et al., 2012; McGuire et al., 2018) Interestingly, CBD seems to act through negative allosteric modulation of CB1R and potentiation of the effects of AEA. (Jakowiecki et al., 2021; Leweke et al., 2012) Both mechanisms would be effective in counteracting the properties of excessive 2-AG signalling.

6.7 TSPO availability in first episode psychosis

In study III, the availability of TSPO was globally lower in first episode psychosis patients in comparison to healthy controls; this is in line with previous results of lower TSPO availability in psychosis compared to controls. (Plaven-Sigray et al., 2018; Plaven-Sigray et al., 2021) Pooled data from first generation TSPO radiotracer studies however revealed increased outcome measure values in psychosis patients than in controls. (Marques et al., 2018) The high likelihood of methodological variability in BP_{ND} estimates of [^{11}C]PK11195, and the use of non-comparable outcome measures in the study published by Marques et al. (2018), unfortunately diminishes the information value which can be gleaned from first generation TSPO radiotracer study meta-analyses, as stated by Plaven-Sigray and Cervenka (2019). The possibility of uptake of a radioactive metabolite of [^{11}C]PK11195 in the brain further complicates interpretations from these studies. (De Vos et al., 1999) Although group differences of non-displaceable binding are also a possible confounder in studies using [^{11}C]PBR28, there does not seem to be a difference in

the distribution volume of non-displaceable binding between first-episode psychosis patients and controls. (Laurell et al., 2021) Thus, the overall picture in the context of first-episode psychosis is that of a lower availability of TSPO than occurs in healthy controls.

6.8 The source of the TSPO signal

The ambiguous source of variation in TSPO's availability, as demonstrated by Notter, Coughlin, Sawa, et al. (2018), hampers attempts at conducting reliable mechanistic interpretations. TSPO was previously viewed as a marker of microglial activation and immune activation. However, it seems increasingly likely that increases in TSPO measures are confined to certain forms of "M1" phenotype-related inflammatory pathophysiology, and that increased microglial inflammatory activity can occur in the absence of elevated TSPO availability. Furthermore, the knowledge that microglia can express variable degrees of both of the simplified "M1" and "M2" phenotypes indicates that the functional role of microglia is affected by the balance of these co-existing phenotypes. The difficulty of interpreting TSPO PET binding in the absence of marked increases of availability is demonstrated by an animal model of poly(I:C)-induced immune activation where TSPO expression was reduced in microglia, astrocytes, as well as in the brain's vasculature. (Notter, Coughlin, Gschwind, et al., 2018) The overall lower expression of TSPO should however be affected to a lesser degree by changes in endothelial cells due to the relatively higher abundance of glial cells. (von Bartheld et al., 2016) On the other hand, neuron activity has been directly associated with levels of TSPO mRNA and protein. (Notter et al., 2021) Lower levels of TSPO in psychoses as compared to controls could thus result from the canonical widespread decreases of frontal cortical neuron activity as seen in schizophrenia.

6.9 The biological implications of altered TSPO functioning

Overall, the previously reported associations of translocator protein functioning to glial cell activity, neuronal activity, and mitochondrial cellular respiration, suggest that individuals suffering from psychosis have globally altered densities, functioning and/or energy metabolism of glial cells and/or neurons. This somewhat vague conclusion should not however be interpreted to mean that the result of decreased TSPO availability is irrelevant. It could reflect a primary underlying pathology of cellular metabolism, or a measurable change which is secondary to dysregulated neurotransmitter systems. For example, mitochondrial dysfunction has been suggested to alter the bioenergetics of the brain, which could very well contribute to

the many neurodevelopmental and state-related phenomenon seen in psychosis. (Whitehurst & Howes, 2022) Associations of medial prefrontal cortex redox status, indexed by glutathione MRS, to TSPO availability suggests that mitochondrial cellular respiration could be altered in psychosis. (Hafizi et al., 2018) It is also possible that the ECS and dopaminergic dysregulation evident in psychoses are compensatory efforts to maintain a normative physiological state in the context of aberrant neurodevelopment due to mitochondrial dysfunction. Interestingly, the availability of TSPO was reported to be elevated in patients with major depression when compared to controls. (Eggerstorfer et al., 2022) The availability of TSPO has been associated to a longer duration of untreated depression, and it also normalized after treatment with antidepressant medication. (Setiawan et al., 2018) TSPO PET measures also seemed to be higher in the context of obsessive-compulsive disorder and substance use disorders. (Meyer et al., 2020) These observations further underline the importance of understanding the mechanisms behind the lower availability of TSPO in psychoses. Unfortunately, the distributions of TSPO availability of psychosis patients and controls overlap considerably, (Plaven-Sigray et al., 2021) and as such, TSPO by itself is not suitable to be used as a diagnostic biomarker.

6.10 Altered peripheral immune signalling in psychotic disorders

In study III, the concentrations of peripheral blood CCL22 were higher in first episode psychosis patients in comparison to matched population controls at baseline, and also after one year of follow-up. The level of CCL22 at baseline was associated to worse cognitive performance and severity of symptoms. High levels of CCL22 predicted persistently high levels of CCL22 at the one-year follow-up. These results indicate the presence of a subgroup of psychosis patients characterized by a chronically altered immune phenotype. An elevation of TNFα was also seen in FEP compared to controls, but the result did not survive the Bonferroni correction for 38 comparisons ($\alpha/m=0.0013$). A meta-analysis conducted by Upthegrove et al. (2014) indicated that the result of elevated TNFα could still represent a true difference between sample populations. This conundrum highlights one problem in studying complex systems, such as the immune system: the functional relevance of a singular result is dependent on the state of the immune system as a whole, but studying the whole system predisposes to spurious erroneous results and correcting for these observations tends to reduce the sensitivity to detect true signals. This issue was tackled in Study II by examining the differences in Spearman rank correlation coefficient networks of FEP and control subjects. This qualitative approach revealed that the profile of the wider cytokine-chemokine signalling system was skewed

towards innate immune signalling and growth factors in first episode psychosis patients when they were compared to controls. The samples were also stratified into high- and low-CCL22 groups to further examine the immune state of FEP subjects with markedly increased levels of CCL22. Although the problem of multiple comparisons is also relevant in this exploration, high-CCL22 FEPs had significantly ($p<0.001$) elevated levels of fractalkine (CX3CL1) and FLT-3L compared to low- and high-CCL22 controls, as well as low-CCL22 FEPs. Fractalkine, acting on the CX3CR1 receptor, functions as a chemoattractant of T-cells and monocytes in the periphery, and as a pro-inflammatory microglial chemoattractant in the brain. Consistently, it has been reported that the CX3CR1 receptor is downregulated in schizophrenia. (Berdenis van Berlekom et al., 2020) CX3CR1 deficient microglia seem to lack neuroprotective functions, which could pivot these cells towards mediating pro-inflammatory actions in the absence of increased TSPO expression. Interestingly, the strong correlation coefficient between fractalkine and TNFα in controls was not seen in FEPs of Study III. CCL22, otherwise known as a macrophage-derived chemokine, is known to be produced by intraparenchymal microglia in addition to peripheral immune cells. (Columba-Cabezas et al., 2002) It is noteworthy that signalling via both fractalkine and FLT-3L has also been associated with innate immune functions.

This observation of a high-inflammatory psychosis subgroup, expressing high levels of CCL22, with plausible connections to established etiological theories of schizophrenia is clearly interesting, but it ultimately raises more questions to be answered. From where is the measured CCL22 secreted, and what is the culprit behind this phenomenon? What is the relationship between altered immune signalling and TSPO expression? What is the relevance of peripheral fractalkine signalling for the brain?

The overall shift of immune signalling towards innate immune functions in psychoses, and the plausible theoretical links between peripheral immune state and the microglia suggest that the immune system is clearly dysregulated in psychotic disorders, and that elucidating the mechanisms behind this phenomenon further could provide new avenues for therapeutic intervention.

6.11 Conclusions

1. The endocannabinoid system of the human adult brain exhibits regionally specific and sexually dimorphic characteristics (I). This is potentially due to sex steroid regulation of the endocannabinoid system. As a result of this, gender should be accounted for in human studies of central ECS function. This sex difference might also have implications for the gender differences in the liability to psychotic and mood disorders.

2. The endocannabinoid system is altered in male first episode psychosis patients in comparison to healthy controls. The distribution volume of the type 1 endocannabinoid receptor was lower in male first episode psychosis patients regardless of whether or not they were using antipsychotic medications. The availability of type 1 endocannabinoid receptor was associated with psychotic symptom scale scores, and performance scores measuring general cognition. The presence of a robust difference of the brain endocannabinoid system functioning, which is also associated with the symptoms of first episode psychosis regardless of medication status, suggests a central etiological role for the endocannabinoid system in psychotic disorders (II).
3. Brain TSPO availability is lower in first-episode psychosis compared to healthy controls matched by age and sex (III).
4. Peripheral immune signalling is consistently altered after first-episode psychosis, and a subgroup of patients with persistently higher CCL22 levels also show evidence of a dysregulation of fractalkine-CX3CR1 signalling (III).

6.12 The strengths and limitations of Studies I-III

Study I did not account for a major gender difference in ECS function, which is why phases of the menstrual cycle were not rigorously documented or accounted for. However, the exclusion criteria applied did allow for ruling out contributions of other confounders, for example effects due to psychiatric disorders, head trauma, cannabis use, alcohol use and smoking. A metabolite corrected arterial plasma input was used for modelling of V_T according to validated methods, which reduced the risk of confounding by differences in radiotracer metabolism or differences in injected dose. The sample size in study I was modest, which increased the risk for a type II error.
Study II was done as an a priori planned simultaneous replication in two geographically different study centers and different sampling populations. This reduced the risk for spurious results. According to power calculations, we had adequate sample sizes for both radiotracers and we accounted for the effects of medication status. However, this was done with samples from clinically slightly different populations. As described for study I, the methodology was validated to be optimal for measuring CB1R availability.

Study III had the same a priori planned concurrent replication design as Study II. We could not account for the effects of medication or diet with regards to the measurements of peripheral cyto- and chemokines. The cyto- and chemokines were measured with the same methodology at the same site, and by using the same assay panels for samples from both recruitment sites. We were unable to synchronize the phase of illness to blood sampling, which potentially induced additional variation

into the data, and possibly contributed to a type I error regarding some of the cyto- and chemokine assays which have been previously associated to specific states of psychotic disorders. While the sample sizes for the cyto- and chemokine measurements were adequate, the sample size for the TSPO PET was modest. As in studies I and II, we used an optimal validated methodology to quantify TSPO's availability. All subjects were genotyped, and the effects of being either MAB or HAB genotype were accounted for by including genotype as a covariate in the statistical models. The PET imaging was fixed to morning imaging slots to rule out the possibility of confounding by any putative diurnal variation of TSPO V_T.

6.13 The main theoretical implications of this thesis

1. A significant sex difference of the endocannabinoid system could potentially represent a major confounder in studies examining the properties of the ECS. Functional and developmental differences could also contribute to the demographic differences in psychiatric disorders between males and females. Furthermore, heightened sensitivity to the possible detrimental effects of cannabis use in either gender should be acknowledged as a possible low-effort prevention strategy to mitigate the population-level effects of increasing cannabis use.
2. Although endocannabinoid receptors can be found on neurons, astrocytes, and microglia, the majority of type 1 receptors has been shown to reside on CCK+ GABA interneurons. Thus, it is probable that a robust decrease in the availability of type 1 endocannabinoid receptors (II) is mostly attributable to functional changes in this neuronal subtype, possibly as a result of changes in the receptor's conformation secondary to increased AEA tonus.
3. The endocannabinoid regulation of glial cells and cellular respiration suggests that the observed changes in the endocannabinoid system in psychosis might be either a driving factor, or a compensatory response, to the proposed changes in cellular respiration and/or immune function in these cells (II and III). Both dysfunctional glial cells, and altered functioning of the endocannabinoid system, might lead to an altered cortical excitation/inhibition balance, disturbed processing of cortical representations, and contribute to the dysfunctional processing of salience.
4. The lower availability of type 1 endocannabinoid receptor (I), and previous observations of elevated anandamide concentrations in the CSF of individuals with psychotic disorders point to the presence of overactive endocannabinoid signalling. This is possibly attributable to increased endocannabinoid synthesis on disinhibited CCK+ GABAergic interneurons, which may

contribute to inhibiting the functions of PV+ interneurons, and subsequently to a disinhibition of cortical glutamatergic projections to the midbrain, as well as evoking mesostriatal dopaminergic hypermetabolism.

5. As stated above, an increased endocannabinoid tonus can act to shift the microglial cells so that they adopt neuroprotective "M2" and surveying "M0" phenotypes. The decreased availability of translocator protein in first episode psychosis (III) supports this proposal. Microglia expressing "M1" phenotype functions play important roles in inflammatory responses, as well as in the elimination of synapses labelled with complement components or neurons expressing proapoptotic signals. The failure to fulfil these roles during development could result in aberrant synaptic connectivity in adulthood. Microglia also exert homeostatic roles as regulators of the extracellular chemical environment, as well as through modulation synaptic structure and function. The failure to exert these actions is an alternative, but not mutually contradicting, hypothesis of how the endocannabinoid system and microglia could interact to contribute to the pathophysiology in psychotic disorders.
6. Increased endocannabinoid signalling can also predispose to excessive lateral synaptic potentiation from astrocyte arrays. Although the dynamics of this mechanism are complex, it can be hypothesized that aberrant potentiation of thousands of neurons can lead to blurring of the boundaries of neural representations, which could in turn lead to a connectivity mismatch and/or a cortical E-I imbalance.

6.14 Directions for future studies

1. The factors behind the sex difference of the endocannabinoid system are unclear. Future studies are needed to further characterize gender differences in the ECS as well as between pre- and postmenopausal women, across different phases of the menstrual cycle, and in the context of hormonal birth control. Human studies on the interactions between gonadal hormones and the endocannabinoid system, as well as animal studies using microdialysis, and comparing the effects of ovarectomy could be ways to facilitate our understanding of the results of Study I.
2. It is necessary to determine whether the endocannabinoid system is implicated in psychotic disorders of females, since this cannot be deduced from the existing data. In future studies examining the properties of the endocannabinoid system in females, it may be necessary to exclude individuals using hormonal contraceptives, and also possibly timing the studies to a specific phase of the menstrual cycle.

3. The different directions of the effects in studies reporting ECS changes in psychotic disorders or gender differences mandate performing a cross-validation study, for example using [^{18}F]FMPEP-*d2* and [^{11}C]OMAR.
4. To assess whether there is a reduction in TSPO availability in psychotic disorders in comparison to controls and whether this is associated to ECS functioning and lower synaptic density, a study should be conducted using both radiotracers for CB1R and measuring synaptic density and TSPO availability.
5. The relationship between blood and CSF endocannabinoids and peripheral immune signalling could be explored.
6. The association of lower CB1R availability to functional and structural dysconnectivity should be evaluated using functional MRI and diffusion weighted imaging. The proposal that alterations in the endocannabinoid system could result in ambiguous borders of neural representations of object categories could be assessed by measuring CB1R availability, and event related potentials both before and after placebo, THC and cannabidiol administration.
7. The role of CCL22 and fractalkine in non-immunological processes and their relevance for psychotic disorders should be more thoroughly characterized.
8. The therapeutic potential of CBD should be clarified. In addition, CBD's mechanisms of action should be elucidated to facilitate further progress in therapeutics, as well as improving our etiological understanding of the ECS.

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