Universitat Autònoma de Barcelona

Characterization of CB_1-5-HT_{2A} receptor heteromers in the olfactory neuroepithelium of cannabis users and schizophrenia patients: implications for clinical and cognitive alterations

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Doctoral Thesis:

Characterization of CB_1-5-HT_{2A} receptor heteromers in the olfactory neuroepithelium of cannabis users and schizophrenia patients: implications for clinical and cognitive alterations

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Date: November 2020


THC
CB1R
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To my family, friends, and mentors


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ABBREVIATIONS

2-AG: 2-arachidonoylglycerol
5-HT: serotonin
5-HT_{2A}R: setotonin receptor type 2A
AC: adenylyl cyclase
ARMS: at risk mental state
cAMP: cyclic AMP
CB_1 /CB_1R: cannabinoid receptor type 1
CB_2 /CB_2R: cannabinoid receptor type 2
CBC: cannabichromene
CBD: cannabidiol
CBG: cannabigerol
CNS: central nervous system
CNVs: copy number variations
COMT: catechol-O-methyltransferase
D_2R: dopamine receptor type 2
DAG: di-acylglycerol
DISC-1: disrupted in Schizophrenia-1
DISC-2: disrupted in Schizophrenia-2
DOI: 2,5-dimethoxy-4-iodoamphetamine
DSM-5: diagnostic and statistical manual of mental disorders – fifth edition
DTNBP1: dystrobrevin-binding protein 1
ECS: endogenous cannabinoid system
FGA: first-generation antipsychotics
GPCR: G protein-coupled receptors
GRIA1: glutamate receptor AMPA 1
GRIN2A: glutamate receptor N-methyl D-aspartate 2A
GWAS: genome-wide association studies
NRG1: neuregulin 1
NSS: neurological soft signs
MAL: myelin and lymphocyte protein
MAP: mitogen-activated protein
MCCB: matrics consensus cognitive battery
ON: olfactory neuroepithelium
PABPC4L*:* poly(A) binding protein cytoplasmic 4-like
PKC: protein kinase C
PLC: phospholipase C
mRNA: messenger ribonucleic acid
SGA: second-generation antipsychotics
SMAD5*:* SMAD family member 5
PET: positron emission tomography
TCF4: gene transcription factor 4
THC: tetrahydrocannabinol
THC-COOH: tetrahydrocannabinol carboxylic acid


THC
CB1R
5-HT2AR

INTRODUCTION

1. Cannabis use in the general population

Cannabis (*Cannabis sativa*, or marijuana) is a plant consisting of multiple cannabinoids. Delta-9-tetrahydrocannabinol (THC) is the primary psychoactive component of cannabis and is responsible for its behavioural and psychotropic effects. There are also non-psychoactive cannabinoids present in cannabis, such as cannabidiol (CBD), cannabichromene (CBC), and cannabigerol (CBG), which have several medicinal properties. Apart from these, the plant also contains other constituents which belong to diverse classes of natural products. To date there are 545 constituents reported in cannabis (Pertwee 2014).

Cannabis is available in multiple preparations. Hashish is created from the resin of marijuana flowers, and it is usually smoked by itself or in a mixture with tobacco, albeit it can also be also ingested. Herbal cannabis (leaves and flowers) and/or oils can be smoked, used to brew tea, or mixed into food products. Cannabis and its related cannabinoids have potential medical applications in the treatment of a variety of serious illnesses (Volkow et al. 2014). More recently however, synthetic cannabinoids have become available as safe legal alternatives to cannabis. These preparations are often more powerful than marijuana and can sometimes be life-threatening (Lafaye et al. 2017). Across all age groups, cannabis is the illicit drug most likely to be used (European Monitoring Centre for Drugs and Drug Addiction 2018), although the legal landscape is changing quickly all over the world, which has been associated to increased usage and reductions in the perception of harm (Cerdá et al. 2017).

1.1. Patterns of cannabis use

The patterns of cannabis use can range from occasional to regular use, but also to drug abuse. It is estimated that 87.7 million European adults aged 15–64 (26.3 %) have experimented with cannabis at some time in their lives. Of these, an estimated 17.1 million young Europeans aged 15–34 (13.9 %), and 10 million aged 15–24 (17.7 %) used cannabis in the last year (European Monitoring Centre for Drugs and Drug Addiction 2018).

Based on general population surveys, it is estimated that around 1 % of European adults are daily or almost daily cannabis users, that is, they have used the drug 20 days or more in the last month (European Monitoring Centre for Drugs and Drug Addiction 2018). Use of cannabis is therefore considered to be frequent (Mounteney et al. 2016) and seems to be increasing (Azofeifa et al. 2016), especially among young people (Malone, Hill, and Rubino 2010). This raises important concerns regarding its possible detrimental effects on cognitive processing during early stages of brain development, and on the increased risk for developing substance use disorders (Malone, Hill, and Rubino 2010), but also psychotic disorders (Di Forti et al. 2019). Overall, the number of first-time treatment-seekers for cannabis-related problems increased from 43.000 in 2006 to 76.000 in 2015. Multiple factors may lie behind this rise, including higher prevalence of cannabis use among the general population, increases in the number of intensive users, the availability of higher potency products, and increases in treatment referral and levels of provision (European Monitoring Centre for Drugs and Drug Addiction 2018).

1.2. The endocannabinoid system

The endogenous cannabinoid system (ECS), encompasses endogenous cannabinoids or endocannabinoids such as N-arachidonoylethanolamide (anandamide) (Devane et al. 1992) and 2-arachidonoylglycerol (2-AG) (Mechoulam et al. 1995), cannabinoid receptors (mainly CB_1R and CB_2R), and the enzymes responsible for the synthesis and degradation of endocannabinoids (Lu and Mackie 2016). Endocannabinoids, unlike classical neurotransmitters, are synthesized 'on demand' from postsynaptic membrane phospholipid precursors (Di Marzo et al. 1994). The ECS is widely expressed in the human brain and is involved in multiple functional processes including emotional regulation, motivational behaviour, and cognitive function (Navarrete et al. 2020), and it can play a crucial role modulating these processes in psychiatric disorders (Koethe et al. 2007; Wong et al. 2010; Katzman, Furtado, and Anand 2016). In addition, the ECS modulates the activity of neurotransmission systems involved in cognition such as glutamate, serotonin (5-hydroxytryptamine or 5-HT), dopamine and GABA (Bloomfield et al. 2019) **(Figure 1)**.

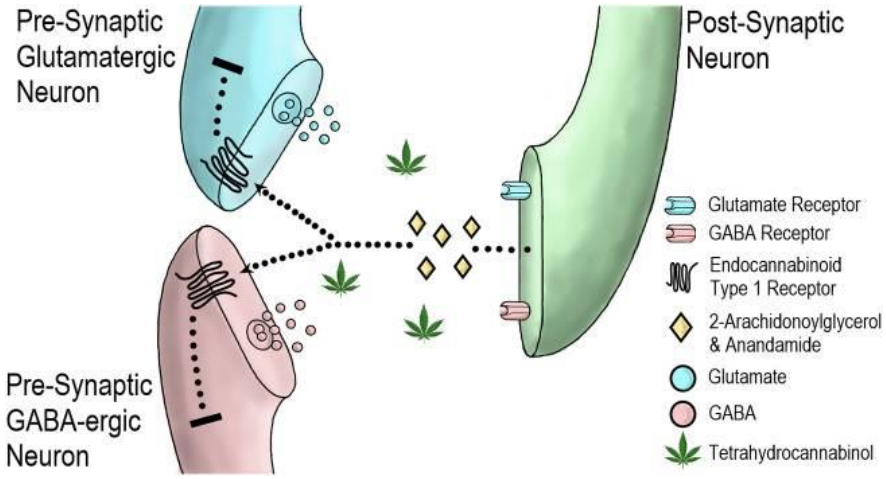

Figure 1. Synaptic cleft showing production of cannabinoids, that upon contact with CB_1R lead to retrograde suppression of excitation in glutamatergic neurons and retrograde suppression of inhibition in GABAergic neurons. THC alters this signalling process by interacting via CB_1R. Image from (Bloomfield et al. 2019).

Further, alterations in CB_1R levels have been described in the brain of cannabis users (Ceccarini et al. 2015; D'Souza et al. 2016; Hirvonen et al. 2012; Mizrahi, Watts, and Tseng 2017) **(Figure 2)**, and changes in endocannabinoid levels are detected in plasma following the administration of THC to healthy volunteers (Thieme et al. 2014; Walter et al. 2013).

1.3. Cannabis use and cognitive effects

Cannabis produces multiple behavioural effects, including reward modulation, changes in sensory perception such as acute paranoia and psychosis, and impaired motor coordination. Other common acute adverse effects of cannabis use include anxiety and panic reactions, often reported by naive users (Hall and Degenhardt 2009). Importantly, chronic cannabis use induces cognitive alterations, including attention, working memory, executive function, and learning deficits (Volkow et al. 2016; Crean et al. 2011; D'Souza et al. 2019; Ford et al. 2017; Solowij and Battisti 2008). These effects are thought to be mediated through its action on CB_1R, and by the modulation of several neurotransmitter systems including 5-HT, glutamate, GABA, and dopamine (Bloomfield et al. 2019). However, the magnitude of the impairments and the extent to which they persist after abstinence are still subject to controversy (Volkow et al. 2016; Schreiner and Dunn 2012).

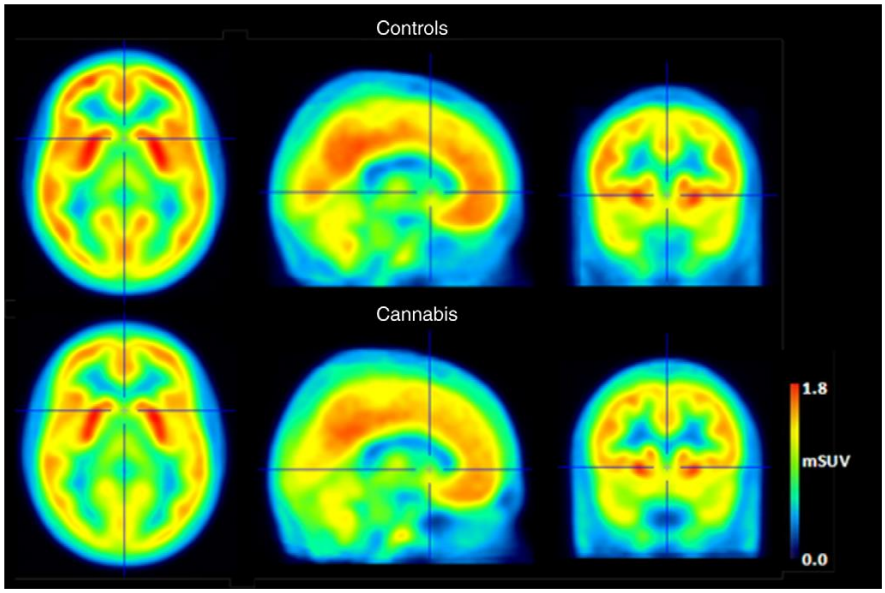

Figure 2. Positron Emission Tomography (PET) image showing selective high-affinity CB_1R PET radioligand [^{18}F]MK-9470 binding *in vivo* in chronic cannabis users and cannabis-naïve controls. Compared with controls, cannabis users showed a global decrease in CB_1 receptor availability, particularly in the temporal lobe, anterior and posterior cingulate cortex, and nucleus accumbens, implying that chronic cannabis use may alter specific regional CB_1R expression through neuroadaptive changes in CB_1R availability. Image from (Ceccarini et al. 2015).

Some studies show that cannabis users present altered structural and functional connectivity in the prefrontal cortex, superior parietal lobe, the hippocampus, corpus callosum and other CB_1R-rich areas of the brain **(Figure 3)** that are important for attention, executive and emotional processing, neural integration, learning and short term memory (Abdullaev et al. 2010; Vaidya et al. 2012; Bloomfield et al. 2019; Lorenzetti, Solowij, and Yücel 2016; Batalla et al. 2013; Filbey and Yezhuvath 2013; Zalesky et al. 2012). Some of these findings are more prominent in patients who started using marijuana in adolescence or young adulthood (Zalesky et al. 2012). However, contradicting data has been put forward after matching by comorbid alcohol use (Weiland et al. 2015), suggesting the possibility that comorbid drug use and/or other underlying conditions could be influencing some of these findings as well.

Nonetheless, approximately 9% of subjects who experiment with marijuana will develop an addictive disorder; this number is higher among those who start using marijuana at a young age and escalates up to 25 to 50% among those who smoke marijuana daily (Volkow et al. 2014).

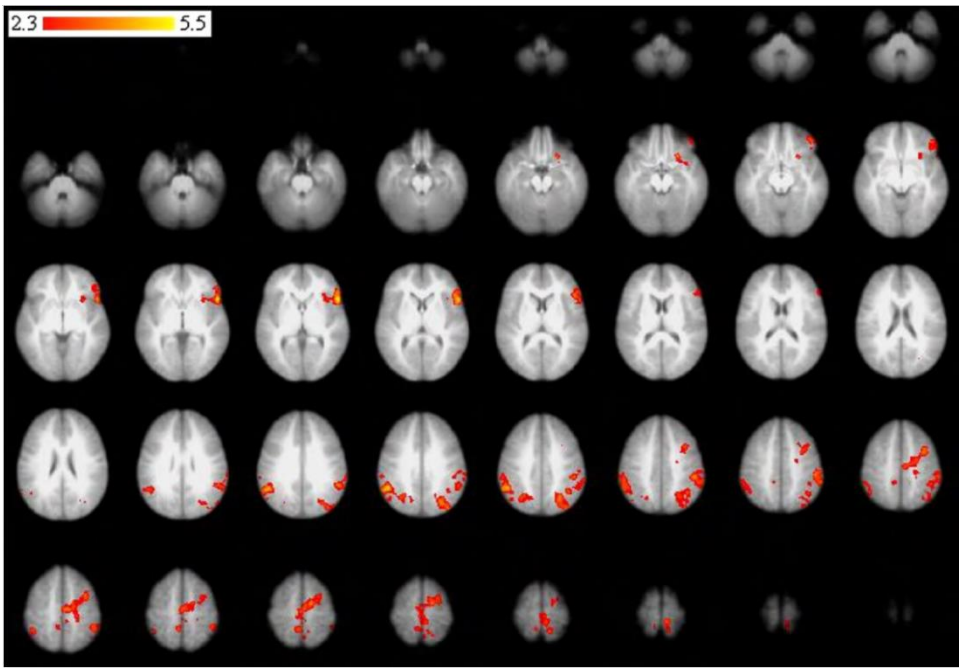

Figure 3. Whole brain functional magnetic resonance images of differences in activation between users and controls during the attention network task, which is a measure of efficiency of attention networks. Compared to the control group, the cannabis user group showed significantly stronger activation in the right prefrontal cortex, supplementary motor cortex and bilateral parietal cortical regions compared to the control group, indicating increased effort to resolve the task. Image from (Abdullaev et al. 2010).

2. Schizophrenia

Schizophrenia is a complex and severe mental disorder that will affect about 7/1000 people, with a higher incidence among immigrants and in urban areas, and with a male: female ratio of about 1.4 (95 percent confidence interval: 0.9, 2.4) (McGrath et al. 2008; Moreno-Küstner, Martín, and Pastor 2018). Schizophrenia causes a significant clinical, familiar and economic burden (Holm et al. 2020; Fusar-Poli, McGorry, and Kane 2017; Chong et al. 2016; Crespo-Facorro et al. 2020), having remained one of the most debilitating disorders in all of medicine (Salomon et al. 2012). In fact, adults with schizophrenia are 2-4 times more likely to die than equivalent adult cohorts in the general population, and present an average decrease in life expectancy of between 15 to 21 years (Tanskanen, Tiihonen, and Taipale 2018; Hayes et al. 2017; Oakley et al. 2018; Laursen, Nordentoft, and Mortensen 2014; Olfson et al. 2015; Hjorthøj et al. 2017).

A leading cause of death in people with schizophrenia is suicide (up to 10%), these numbers being higher for men and during the initial phases of the illness (Saha, Chant, and McGrath 2007; Popovic et al. 2014; Hor and Taylor 2010; Thomas Munk Laursen, Nordentoft, and Mortensen 2014; Tanskanen, Tiihonen, and Taipale 2018), which is aggravated by a lack of access to adequate mental healthcare care (Schoenbaum et al. 2017) and the presence of neuropsychiatric comorbidities (Hunt et al. 2018; Plana-Ripoll et al. 2019). Other causes of death include metabolic/cardiovascular, respiratory, and infectious, as well as accidents and cancer (Walker, McGee, and Druss 2015; Hayes et al. 2017; Bitter et al. 2017; Laursen, Munk-Olsen, and Vestergaard 2012; Lee et al. 2018; Tanskanen, Tiihonen, and Taipale 2018; Oakley et al. 2018; Olfson et al. 2015; Hjorthøj et al. 2017). These fatalities may be related to factors such as obesity, diabetes, hyperlipidaemia, higher prevalence of nicotine use, reduced engagement in healthy lifestyle, and disparities in access to medical and preventive care, such as cancer screening (DE Hert et al. 2011; Moore et al. 2015; Farrer et al. 2018; Björk Brämberg et al. 2018; Momen et al. 2020; Kugathasan et al. 2020; 2018; Solmi et al. 2020).

2.1. Clinical and functional aspects of schizophrenia

Schizophrenia is characterized by a heterogeneous constellation of symptoms such as hallucinations, which are perceptual or sensory alterations occurring without apparent causing stimuli; delusions, which are beliefs or ideas that are strongly held and not amenable to change in light of conflicting evidence; and disorganized thought and speech, ranging from easy topic-switching (derailment or loose associations), to nearly incomprehensible discourse (incoherence)(American Psychiatric Association 2013), all of which are known globally as positive symptoms. The diagnostic criteria for schizophrenia according to the Diagnostic and Statistical Manual of Mental Disorders – Fifth Edition (DSM-5) (American Psychiatric Association 2013) are listed in **Table 1**.

Additionally, schizophrenia is characterized by negative symptoms, which encompass a diverse array of signs and symptoms that include avolition (reduced initiation and persistence of goal-directed activity), alogia (reduction in the quantity of speech and in its spontaneous elaboration), anhedonia (diminished capacity to experience pleasant

emotions), affective flattening (decrease in the observed expression of emotion), and asociality (reduction in social initiative due to decreased interest in forming close relationships) (Marder and Galderisi 2017; Kirkpatrick et al. 2006). Primary negative symptoms are a core feature of schizophrenia, but their underlying etiopathogenetic mechanisms are still unknown (Mucci et al. 2017), whereas secondary negative symptoms are transient in nature and are attributable to other factors such as positive symptoms, depression, medication side-effects, social deprivation or substance abuse, and they often subside with resolution of the causative factors (Kirschner, Aleman, and Kaiser 2017). Negative symptoms are common, with reported prevalence of 19-37% (Buchanan 2007), with more than half of the patients displaying at least two types of negative symptoms (Bobes et al. 2010). Furthermore, negative symptoms account for much of the long-term disability and poor health and functional outcomes of patients with schizophrenia (Ventura et al. 2015; Fervaha et al. 2014; Bowie et al. 2008; Evensen et al. 2012; Rabinowitz et al. 2012).

Table 1. DSM-5 diagnostic criteria for schizophrenia

Criterion A. Two (or more) of the following, each present for a significant portion of time during a 1 -month period (or less if successfully treated). At least one of these must be (1), (2), or (3):
1. Delusions 2. Hallucinations 3. Disorganized speech (e.g., frequent derailment or incoherence) 4. Grossly disorganized or catatonic behavior 5. Negative symptoms (i.e.: diminished emotional expression or avolition)
Criterion B. For a significant portion of the time since the onset of the disturbance, level of functioning in one or more major areas, such as work, interpersonal relations, or self-care, is markedly below the level achieved prior to the onset (or when the onset is in childhood or adolescence, there is failure to achieve expected level of interpersonal, academic, or occupational functioning).
Criterion C. Continuous signs of the disturbance persist for at least 6 months. This 6-month period must include at least 1 month of symptoms (or less if successfully treated) that meet Criterion A (i.e., active-phase symptoms) and may include periods of prodromal or residual symptoms. During these prodromal or residual periods, the signs of the disturbance may be manifested by only negative symptoms or by two or more symptoms listed in Criterion A present in an attenuated form (e.g., odd beliefs, unusual perceptual experiences).

Table 1. DSM-5 diagnostic criteria for schizophrenia (continued)

Criterion D. Schizoaffective disorder and depressive or bipolar disorder with psychotic features have been ruled out because either 1) no major depressive or manic episodes have occurred concurrently with the active-phase symptoms, or 2) if mood episodes have occurred during active-phase symptoms, they have been present for a minority of the total duration of the active and residual periods of the illness.
Criterion E. The disturbance is not attributable to the physiological effects of a substance (e.g., a drug of abuse, a medication) or another medical condition.
Criterion F. If there is a history of autism spectrum disorder or a communication disorder of childhood onset, the additional diagnosis of schizophrenia is made only if prominent delusions or hallucinations, in addition to the other required symptoms of schizophrenia, are also present for at least 1 month (or less if successfully treated).

2.2. Neurological Soft Signs

Neurological soft signs (NSS) are subtle but observable impairments in simple motor coordination, complex motor sequencing, sensory integration and disinhibition signs that are not localized to a specific area of the brain nor characteristic of any specific neurological condition (Bombin, Arango, and Buchanan 2005; Chan and Gottesman 2008). It is generally accepted that NSS are more prevalent in schizophrenia patients compared to healthy subjects.

NSS are present prior to the start of treatment and are believed to be independent of illness phase (as well as type of antipsychotic treatment) (Gourion et al. 2004; Bombin, Arango, and Buchanan 2005; Chan et al. 2010). In fact, they have been consistently demonstrated in neuroleptic-naïve first-episode patients, i.e. prior to medication exposure, supporting the assumption that NSS constitute an intrinsic feature of schizophrenia (Bachmann and Schroder 2017; Dazzan and Murray 2002; Chan et al. 2010). NSS scores have been proposed as course predictors since they decrease in the clinical course of schizophrenia with remission of psychopathological symptoms.

However, NSS have also been associated with more chronic and severe forms of the illness (Whitty et al. 2003; Bachmann and Schroder 2018). NSS have been significantly associated with baseline positive and negative symptoms, and with the prospective response to antipsychotic treatment (Mittal et al. 2007).

In untreated patients, NSS seem to be associated with symptom severity, and elevated NSS are predictive of fewer improvement in symptoms after antipsychotic treatment (Bachmann and Schroder 2018). These findings are consistent with the hypothesis that NSS are linked to the neuropathology that underlies schizophrenia symptomatology and course (Neelam, Garg, and Marshall 2011; Varambally, Venkatasubramanian, and Gangadhar 2012).

2.3. Cognitive Deficits in Schizophrenia

Schizophrenia is also characterized by cognitive deficits that expand across all cognitive domains, particularly in attention, verbal memory, speed of processing, and working memory (Fatouros-Bergman et al. 2014; Mesholam-Gately et al. 2009), although with significant individual variation (Kremen et al. 2004; Allen, Goldstein, and Warnick 2003; Rodríguez-Sánchez et al. 2020).

Evidence accumulates pointing towards the fact that cognitive alterations seem to be a distinct symptom domain of schizophrenia that is present across the course of the illness, and that independently predicts long-term functional outcome and quality of life (Keefe 2014; Mesholam-Gately et al. 2009; Kane and Lencz 2008; Keefe and Fenton 2007; Green, Kern, and Heaton 2004; Sponheim et al. 2010). However, despite the clinical and functional importance of cognitive symptoms, there are no currently approved pharmacologic treatments for cognitive alterations (Harvey and Keefe 2001; Coyle et al. 2010; Menniti et al. 2013; Choi, Wykes, and Kurtz 2013), partly due to the lack of specific biomarkers that could guide drug development.

Functional connectivity, white matter integrity and structural alterations have been related to cognitive symptoms in schizophrenia (Lesh et al. 2011; Sheffield and Barch 2016; Castro-de-Araujo et al. 2018; Penadés et al. 2019; Dienel and Lewis 2019) **(Figure 4)**, but the field is currently far from reaching a consensus about causative factors. Consequently, the specific neurobiological mechanisms underlying cognitive symptoms in schizophrenia remain elusive, and so does their interaction with environmental factors such as stress, psychological trauma, or drug use. Thus, the need to search for relevant biomarkers that could inform the neurobiology of cognitive symptoms and their interaction with environmental factors in schizophrenia is pressing.

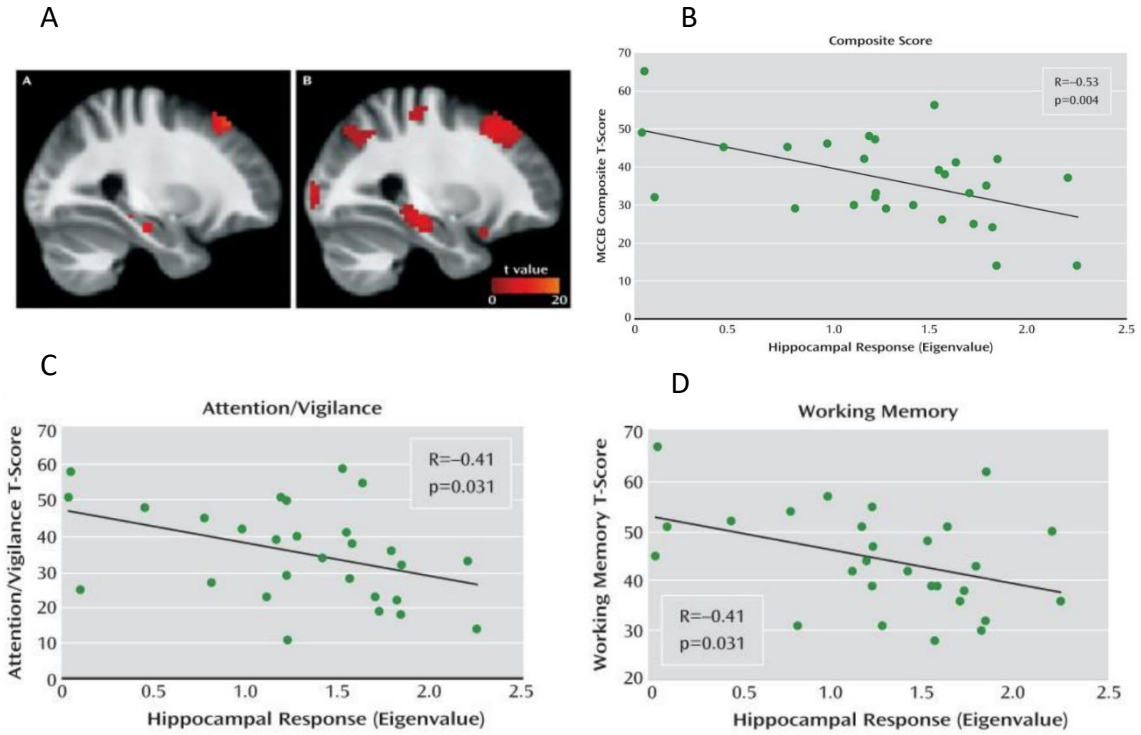

Figure 4. Associations between cognition measures and intrinsic hippocampal activity in schizophrenia patients, (A) Significantly different intrinsic resting activity as measured by functional magnetic resonance imaging in healthy controls (panel A) and patients with schizophrenia (panel B). (B).On the same sample, significant association between cognition, as measured by the composite score on the MATRICS Consensus Cognitive Battery (MCCB) and intrinsic hippocampal activity in patients. Exploratory correlation analyses revealed that the effect was driven by negative associations between intrinsic resting hippocampal activity and the MCCB domains of attention/vigilance (C) and working memory (D). Image adapted from (Tregellas et al. 2014).

2.4. Genetic correlates of schizophrenia

Based on twin and family studies, heritable factors are estimated to explain 80% of the risk of developing schizophrenia (Marder and Cannon 2019). Large-scale genome-wide association studies (GWAS) have allowed for simultaneous study of millions of single nucleotide polymorphisms (SNP) across all of the genome, and seem to point to a polygenic risk profile for schizophrenia, implicating multiple loci, albeit with relatively small individual effects on risk (Ripke et al. 2013; Rammos et al. 2019; Purcell et al. 2009). Other alterations known as rare copy number variations (CNV) are much more infrequent but have larger effect sizes (Purcell et al. 2014; Genovese et al. 2016).

More specifically, possible candidate genes for susceptibility to schizophrenia include the Disrupted in Schizophrenia (DISC)-1 and DISC-2 genes, as well as, 5-HT_{2A} and D3 receptor coding genes, the dopamine transporter (SCL6A3) and for catechol-O-methyltransferase (COMT), which is the enzyme that metabolizes dopamine, or the gene transcription factor 4 (TCF4). Variations have also been found in genes related to glutamate receptors such as N-methyl D-aspartate 2A (GRIN2A) or AMPA 1 (GRIA1), or to synaptic plasticity, such as dystrobrevin-binding protein 1 (DTNBP1), neuregulin 1 (NRG1), or to myelination processes, such as the myelin and lymphocyte protein (MAL) (Miyamoto et al. 2003; Rammos et al. 2019; van de Leemput et al. 2016; Vázquez-Bourgon et al. 2014). These data point towards widespread alterations affecting multiple neurotransmitter systems, synaptic plasticity and function, cytoskeletal development, and the immune system (Radhakrishnan, Kaser, and Guloksuz 2017; Bennett 2011).

Despite the many advances reported in preclinical, genetic and neuroimage studies, however, a clear link between etiology, pathophysiology and biological processes, and its relation to specific behavioural or cognitive symptoms remains largely to be traced in schizophrenia (Flores, Morales-Medina, and Diaz 2016; Potkin et al. 2010; van der Merwe et al. 2019; Howes et al. 2017). This is due to a variety of factors, including the challenge of obtaining viable neurological tissue for the study of its underlying neurophysiological processes.

Moreover, environmental factors, including obstetrical complications, early-life adversity and trauma, urban residence, and drug use, probably interact with underlying genetic risks to influence risk of developing schizophrenia (Marder and Cannon 2019).

2.5. Cannabis use and vulnerability to schizophrenia

A positive, dose-dependent association between cannabis use and the risk of schizophrenia has been documented repeatedly in epidemiological studies, particularly in young people with high rates of cannabis use (Marconi et al. 2016; Gage, Hickman, and Zammit 2016). Prevalence of cannabis use is 2-4 times higher in patients who have experienced a first psychotic episode than in the general population (Bergé et al. 2016; Jónsdóttir et al. 2013) and the risk of psychosis onset is 2.6 times greater in cohorts of cannabis users and tends to appear earlier, particularly in cases of heavier marijuana use, higher drug potency/strength and exposure at younger age (Moore et al. 2007; Mané et al. 2017; Di Forti et al. 2014).

The apparent detrimental effect of cannabis in schizophrenia continues during the course of illness, whereby generally lower treatment adherence and higher risk of relapse are reported among patients who continue cannabis use (Bergé et al. 2016; Linszen, Dingemans, and Lenior 1994). These findings are supported by molecular and genetic studies highlighting the role of cannabis in altering various neurotransmission pathways linked to the pathogenesis of psychotic disorders and by interfering with neurodevelopment (Sherif et al. 2016; Vaucher et al. 2018).

However, a causal relationship between cannabis use and psychotic disorders remains elusive because randomized controlled trials exposing at-risk subjects to cannabis are ethically unacceptable and observational findings can always be hampered by confounding factors (i.e. an unknown risk factor associated to cannabis, instead of cannabis, causes the disease), and/or reverse causality (i.e. schizophrenia patients may be more disposed and/or vulnerable to use cannabis) (Petersen et al. 2019; Vaucher et al. 2018).

Along those lines, previous studies have shown that genetic risk factors for cannabis use and schizophrenia are positively correlated (Power et al. 2014; Verweij et al. 2017), and the coexistence of both disorders generally worsens severity and prognosis (Torrens et al. 2012; Stone et al. 2014).

2.6. Treatment of schizophrenia

Antipsychotic medications are the cornerstone of successful acute treatment and of relapse prevention in schizophrenia (Buchanan et al. 2010; Leucht et al. 2012; Keating et al. 2017; Huhn et al. 2019; Crespo-Facorro, Pelayo-Teran, and Mayoral-van Son 2016). The purpose of acute treatment with antipsychotic drug therapy is to alleviate acute symptoms in order to get the patient back to his or her baseline functioning, whereas maintenance therapy will seek to avoid relapse of symptoms and thus maximize functioning and quality of life (Keepers et al. 2020). Antipsychotic monotherapy should be preferred, although a subgroup of patients could benefit from antipsychotic polytherapy (Guinart and Correll 2020).

Antipsychotic medications are usually classified as typical, also known as first-generation antipsychotics (FGA), and atypical, or second-generation antipsychotics (SGA) due to their diverse pharmacodynamic profile. Unlike FGAs, SGAs show less specific D_2R antagonism and often a relatively stronger 5-$HT_{2A}R$ antagonism, as well as adrenergic, antihistaminergic, and anticholinergic activities (Correll 2010). Thus, of relevance to this thesis, 5-$HT_{2A}R$ plays an important role in both the therapeutic and side-effect profile of antipsychotics (Miyamoto et al. 2005; Correll 2010).

The degree of clinical response to antipsychotics varies widely (Marder and Cannon 2019). Up to 80% of patients will respond to the first treatment with an antipsychotic medication by 4 weeks to 1 year. However, between 10 and 30% will have limited response, and some will have persistent psychotic or residual symptoms that affect their functioning and their quality of life (Kane et al. 2019).

Of note, response rates usually refer to positive symptoms, such as delusions or hallucinations, which tend to respond better to D_2R blockade, particularly when the levels of antipsychotic medication in the central nervous system (CNS) are sufficient to occupy approximately 70% of D_2Rs (Uchida et al. 2011).

However, as discussed in earlier sections, there are not currently approved pharmacologic treatments for both negative and cognitive symptoms of schizophrenia. In fact, among other side effects (Solmi et al. 2017), both FGAs and SGAs can worsen cognitive functions in first episode schizophrenia, albeit FGAs seemingly more intensely (Zhang et al. 2013), probably due to strong D_2R blockade (Sakurai et al. 2013). On the other hand, cognitive function benefits indirectly from an improvement in hallucinations and thought disorganization (Trampush et al. 2015), which is achieved thanks to antipsychotic medication, so the net effect of antipsychotics on cognition seems to be close to zero (Solmi et al. 2017).

3. Role of the serotoninergic system in schizophrenia

Serotinin (5-HT) is a compound found primarily in the CNS, but also in the gastrointestinal tract and blood platelets. It mediates multiple physiological and regulatory functions including sleep cycle, gastrointestinal motility, haemostasis, thermoregulation, and vascular integrity (Cryan et al. 2000; Portas, Bjorvatn, and Ursin 2000; Calabrò et al. 2019; Magalhães et al. 2010). In the human brain, 5-HT is synthesized in the brainstem's raphe nuclei and is projected through synaptic connections into the rest of the brain, other brainstem nuclei and the spinal cord, also through paracrine transmission (Hornung 2003; Pollak Dorocic et al. 2014), which allows 5-HT to regulate such a diverse array of bodily functions. 5-HT receptors have been classified into seven subgroups ($5\text{-}HT_1$ to $5\text{-}HT_7$), although a diverse number of subtypes and variants exist, and importantly, 5-HT receptors have shown operational diversity, as well as potential to form homo or heteromers (Hannon and Hoyer 2008; Hoyer, Hannon, and Martin 2002; Beliveau et al. 2017).

Due to its multiple regulatory roles in bodily homeostasis and basic biological functions, it is not surprising that disturbances in the 5-HT system have been linked to a diverse range of neuropsychiatric disorders such as anxiety, impulsivity, major depression, and schizophrenia (Hornung 2003; Miyazaki, Miyazaki, and Doya 2012; Stępnicki, Kondej, and Kaczor 2018; Müller and Jacobs 2010). Thus, stress-related 5-HT hypersecretion originating in the dorsal raphe nucleus could cause disruption in the activity of cortical neurons in the anterior cingulate and dorsolateral frontal cortices via removal of phospholipids from the cell membrane as part of a signalling cascade, a process that could lead to some of the symptoms seen in schizophrenia (Eggers 2012; 2013).

3.1. 5-HT$_{2A}$ receptors and schizophrenia

5-HT$_{2A}$R are widely expressed in the human brain across both cortical and subcortical regions, with higher density than other receptors from the same neurotransmitter family, as shown in **Figure 5**.

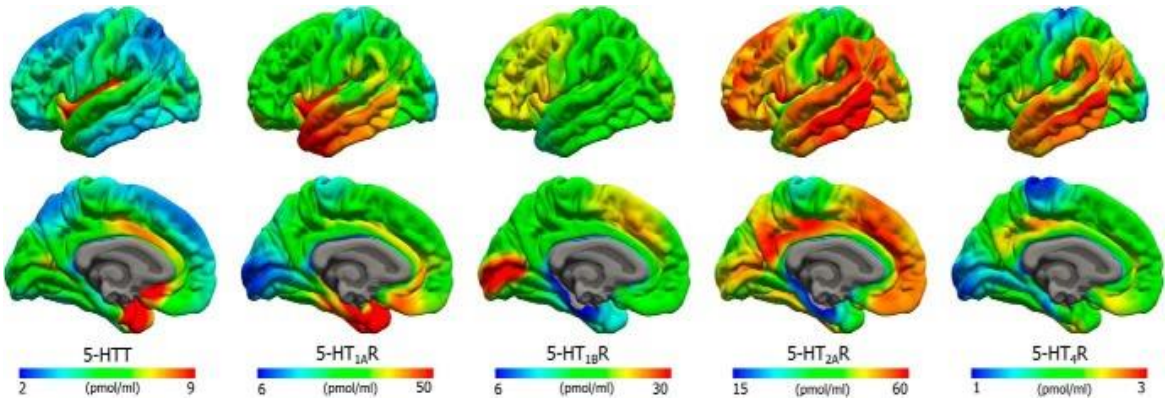

Figure 5. Average density of expression of different serotonergic receptors in the human brain; left hemisphere. Upper row shows a lateral view of the cortical surface, the bottom row shows a medial view. Image from (Beliveau et al. 2017).

Evidence of the involvement of 5-HT_{2A}R in schizophrenia has accumulated in recent years. For instance, hallucinatory drugs such as lysergic acid diethylamide (LSD), mescaline or psilocybin, all have in common a high affinity for 5-HT_{2A}R (López-Giménez and González-Maeso 2018), and 5-HT_{2A}R agonists worsen symptoms in mouse models of schizophrenia (Santini et al. 2013).

These findings, together with the fact that atypical or second-generation antipsychotics, which are a core feature of the treatment of schizophrenia are essentially 5HT_{2A}R antagonists (Meltzer and Massey 2011; Kusumi, Boku, and Takahashi 2015; Miyamoto et al. 2005) strongly suggest the involvement of 5HT_{2A}R in the pathogenesis of schizophrenia. Further, polymorphisms in genes coding this receptor have been associated with a higher risk for schizophrenia (Golimbet et al. 2007; Miyamoto et al. 2003) and have been related to characteristic dysregulations in sensory information processing in schizophrenia (Quednow et al. 2008).

Using radioligands, a recent study found that 5-HT_{2A}R are up-regulated in postmortem frontal cortex of antipsychotic-free schizophrenic subjects, but not in patients treated with antipsychotics (Muguruza, Moreno, et al. 2013). Conversely, in vivo molecular imaging studies show significantly lower 5-HT_{2A}R binding in the frontal cortex of treatment-naïve schizophrenia patients, which correlated negatively to positive psychotic symptoms, but only in the male patients (Rasmussen et al. 2010). In this study, no relationship to cognitive symptoms was found, but the sample size was small. A recent meta-analysis tried to tackle these inconsistencies including both post-mortem and molecular imaging studies, and reported a reduction in prefrontal 5-HT_{2A}R with a large effect size pooling together 168 patients and 163 controls, albeit with substantial heterogeneity between studies (Selvaraj et al. 2014).

More recently, an in vivo imaging study in schizophrenia patients treated with clinically relevant doses of second-generation antipsychotics (olanzapine, risperidone, aripiprazole and quetiapine), showed that 5-HT_{2A}R availability was lowered by antipsychotic use, albeit unevenly (Radhakrishnan et al. 2020).

Finally, the presence of a dysfunctional 5-HT_{2A}R conformation with pro-hallucinogenic features has been put forward in the dorsolateral prefrontal cortex of subjects with schizophrenia, which would also be modulated by antipsychotic treatment (García-Bea et al. 2019).

Interestingly, 5-HT_{2A}R expression alterations have also been found in the brain of at risk mental state (ARMS) subjects, which can be considered as a continuum of incremental morbidity that eventually culminates in first-episode psychosis (Hurlemann et al. 2008), highlighting the fact that alterations in the 5-HT_{2A}R could be present before illness onset. Together, these findings suggest that frontal cortical 5-HT_{2A}R could be involved in the pathophysiology of schizophrenia.

However, the specific role of $5HT_{2A}$ receptors in the different functions, signs and symptoms of schizophrenia is, at best, only known to a certain extent, and even less so the potential role that variations in receptor structure, involvement of additional players and/or complex heterodimer formation may have in schizophrenia. Also, whether such variations occur in response to or are modulated by exogenous substances, like cannabis remains to be elucidated.

4. Role of the endocannabinoid system in schizophrenia

In schizophrenia, post-mortem studies have consistently reported an increase in CB_1R density in the the dorsolateral prefrontal cortex (Dalton et al. 2011; Jenko et al. 2012; Volk et al. 2014). In addition, other post-mortem brain radioligand binding studies have shown increased density of CB_1R in the anterior (Zavitsanou, Garrick, and Huang 2004) and posterior (Newell, Deng, and Huang 2006) cingulate cortices. Conversely, a series of immunoreactivity studies show CB_1R to be seemingly decreased in the postmortem dorsolateral prefrontal cortex of schizophrenic subjects compared to controls (Eggan, Hashimoto, and Lewis 2008; Eggan et al. 2010; Urigüen et al. 2009). Further, while some in vivo PET scans with radiotracer show decreased availability of CB_1R in multiple subcortical regions, as well as the posterior cingulate cortex (Ranganathan et al. 2016), others show increased CB_1R availability in the cingulate cortex and other subcortical regions (Ceccarini et al. 2013), including the brain stem and pons (Wong et al. 2010).

Lastly, while some studies report a significant decrease in CB_1R mRNA levels in the prefrontal cortex of schizophrenia patients (Eggan, Hashimoto, and Lewis 2008; Muguruza et al. 2019), others do not (Dalton et al. 2011; Urigüen et al. 2009). Many methodological issues could account for such discrepant findings, as well as treatment with antipsychotic medication, but overall, while seemingly inconclusive, imaging and postmortem research on the availability, density and expression of CB_1R in schizophrenia point towards a relevant role of this receptor that needs to be further elucidated (Inés Ibarra-Lecue et al. 2018).

Other studies in post-mortem samples have shown altered levels of endocannabinoids in several brain structures (Muguruza, Lehtonen, et al. 2013). Thus, schizophrenia patients showed higher 2-AG levels in the cerebellum, hippocampus and prefrontal cortex when compared to matched controls, whereas anandamide levels were lower in all three regions. Additionally, lower levels of docosahexaenoylethanolamine were also found in cerebellum and hippocampus, but not in the prefrontal cortex. Finally, levels of cannabimimetic compounds, such as palmitoyl-ethanolamine and dihomo-γ-linolenoylethanolamine were also found to be lower in the cerebellum (Muguruza, Lehtonen, et al. 2013).

As for the enzymes involved in the synthesis and degradation of endocannabinoids, the levels of the anandamide degrading enzyme, fatty acid amide hydrolase, were shown to be altered in the cortex of schizophrenic patients (Muguruza et al. 2019). In addition, a diminished production of NAPE and DAGL, enzymes involved in endocannabinoid synthesis, has been reported in plasma of schizophrenia patients (Bioque et al. 2016), which correlated with lower scores in working and verbal memory. Even though blood levels do not necessarily reflect changes within the CNS, these data highlight the potential role of the ECS in the cognitive alterations in schizophrenia. Taken together, these findings seem to reveal an imbalance in the expression and function of different elements of the endocannabinoid system in schizophrenia.

5. Interaction between the endocannabinoid and serotoninergic systems

In rodent studies it has been demonstrated that CB_1R are expressed in central serotoninergic neurons, and a bilateral interaction between the serotonergic and endocannabinoid systems has been revealed (Häring et al. 2015). On the one hand, 5-HT could play a role in the ability of CB_1R to couple to its G-protein second messenger system, and thus launch intracellular signalling (Devlin and Christopoulos 2002). Further, chronic treatment with selective 5-HT reuptake inhibitors, which elevate synaptic 5-HT concentrations, induces hypersensitization of GTPS signalling elicited by CB_1R activation, and also increases CB_1R density in prefrontal cortex (Umathe, Manna, and Jain 2011). Also, activation of $5\text{-}HT_{2A}R$ induces 2-AG release in rodents (Parrish and Nichols 2006).

Conversely, it has been demonstrated that cannabinoid receptor agonists can specifically upregulate $5\text{-}HT_{2A}R$ (Franklin and Carrasco 2013). Moreover, functional CB_1R signaling in 5-HT neurons modulates dinstinct 5-HT-mediated behaviors in mice. Thus, mice lacking the CB_1R receptor in serotonergic neurons are more anxious and less sociable than control littermates (Häring et al. 2015). Interestingly, other studies have reported that the administration of the $5\text{-}HT_{2A}R$ agonist, the hallucinogen 2,5.-dimethoxy-4-iodoamphetamine (DOI) induces a head-twitch response, and this effect is modulated by endocannabinoids (Darmani 2001; Ibarra-Lecue et al. 2018), supporting an interaction between the serotoninergic and the endocannabinoid systems in psychotic-like behaviour in rodents.

All these findings indicate the presence of underlying functional interactions between the endocannabinoid and serotoninergic systems (Haj-Dahmane and Shen 2011), which may be clinically relevant in humans. Although human studies have shown that the ECS may be involved in the regulation of mood and stress-related behavioural alterations observed in recreational cannabis users (Williamson and Evans 2000), there is a paucity of data relating and interaction between these two systems.

Hence, up to date, to our knowledge, there is only one study showing that activation of 5-HT_{2A}R induces endocannabinoid release (Dos Santos et al. 2018). Therefore, there is a pressing need to further characterize the specific cellular mechanisms of this crosstalk, and the interaction with exogenous elements.

5.1. CB_1-5-HT_{2A} receptor heteromers and their signalling properties

G protein-coupled receptors (GPCR) can form heteromer complexes through receptor oligomerization in the CNS (Fuxe et al. 1983; Ferré et al. 2014). Interestingly, the dysfunction or disruption of GPCR heterodimer complexes has been described as a potential molecular basis for potential pathological changes in brain circuits leading to neuropsychiatric diseases including schizophrenia (Borroto-Escuela et al. 2017).

Both 5-HT_{2A}R and CB_1R are GPCR. The 5-HT_{2A}R activates phospholipase-C (PLC) through G_q and leads to an accumulation of IP_3, di-acylglycerol (DAG), and activation of protein kinase C (PKC). Inside the cytoplasma, a rise in IP_3 causes the intracellular endoplasmic reticulum stores to release calcium, an action shared with other GPCRs (Raote et al. 2007). In contrast, the CB_1R couples to multiple intracellular signalling pathways, including the canonical inhibitory Gi/o-proteins, whose activation inhibits adenylyl cyclase (AC), and thus production of cyclic AMP (cAMP), as well as other G-protein subtypes ($G_{s/q}$), and β-arrestins coupled to mitogen-activated protein (MAP) kinases (Howlett, Blume, and Dalton 2010; Priestley, Glass, and Kendall 2017).

Recently, it has been described that CB_1R and 5-HT_{2A}R form heteromers that are expressed and functionally active in the brain of mice, where they specifically mediate the memory impairment induced by THC (Vinals et al. 2015). This newly characterized CB_1R-5-HT_{2A}R heteromer in mice displayed negative crosstalk and bidirectional cross-antagonism, meaning that co-stimulation by agonists reduces intracellular signalling, whereas antagonist binding to one of the receptor subunits blocks the signalling of the other subunit/interacting receptor (**Figure 6**; see (Vinals et al. 2015) for details).

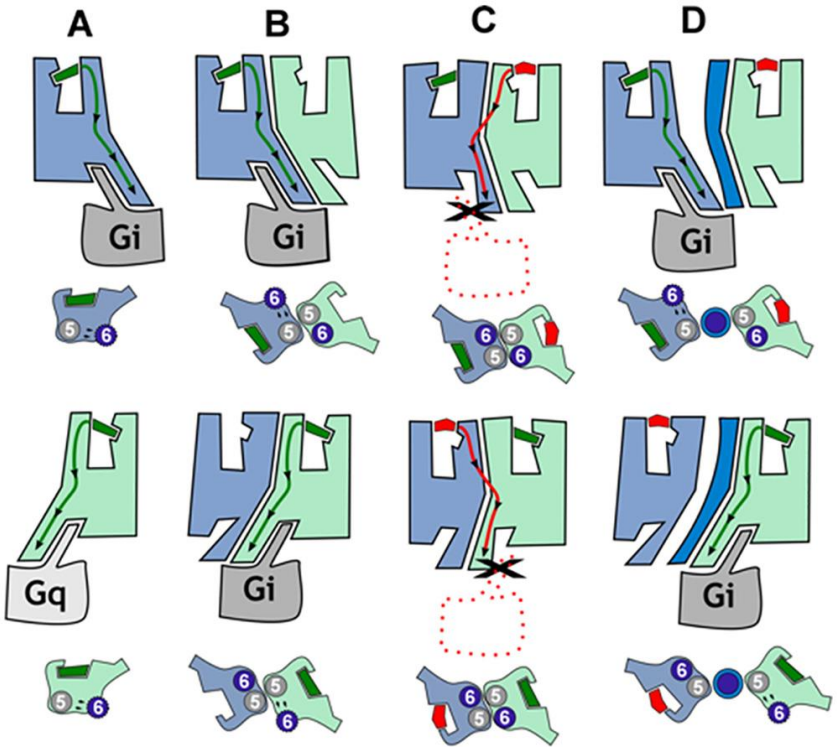

Figure 6. Proposed functional properties of CB_1R-5-$HT_{2A}R$ heteromers. In (A), agonist binding to CB_1R (blue) or 5-$HT_{2A}R$ (light green) triggers the conformational changes of TMs 5 and 6, opening the intracellular cavity for Gi and Gq binding, respectively. In (B), the formation of the CB_1R-5-$HT_{2A}R$ heteromer makes both receptors signal via Gi. In (C), rimonabant binding to CB_1R or MDL 100,907 to 5-$HT_{2A}R$ stabilizes the closed conformation of the receptor, facilitating heterodimerization via TMs 5 and 6 as in the crystal structure of the μ-opioid receptor. In this assembly, both protomers are locked in the closed conformation since the opening of TMs 5 and 6 for G-protein binding is not feasible. Bidirectional cross antagonism is due to the fact that antagonist binding to any protomer must, in addition to its common role in a monomeric signaling unit, disrupt this very stable four-helix association. (D) In agreement, bidirectional cross antagonism is abrogated following treatment with TM 5 or TM 6 interference peptides (dark blue), which disrupt the heteromer structure. Image from (Vinals et al. 2015).

Given the above-described findings, it is possible that a CB_1R and 5-$HT_{2A}R$ interact via the formation of a heterodimer complex in humans too, and that it could play a role in the pathophysiology of schizophrenia. However, the presence and functionality of this CB_1R-5-$HT_{2A}R$ heterodimer in humans affected with schizophrenia, as well as its clinical correlates remain to be characterized. Also, there is no evidence regarding how cannabis use may modulate the signalling properties of this heteromer in healthy controls or in schizophrenia patients. All of these questions are objectives of the present thesis work.

6. The olfactory neuroepithelium to study biomarkers of schizophrenia

One of the most prominent challenges in neuropsychiatric research is the lack of safe, direct access to the brain of a living human. In a context of increasingly productive collaborations to enhance translational research in neuropsychiatric disorders, the study of the olfactory neuroepithelium (ON) has recently emerged as a promising alternative to help characterize biomarkers and study the molecular mechanisms involved in the pathogenesis of several neuropsychiatric disorders (Lavoie et al. 2017; Horiuchi et al. 2013).

The ON is located dorsally and posteriorly inside the nasal cavity and is composed of different cell types including basal cells (progenitor cells), olfactory neurons (mature and immature), and supporting cells. The ON is connected to the olfactory bulb through the lamina propria and the cribriform plate or lamina cribosa (Sawa and Cascella 2009) **(Figure 7).**

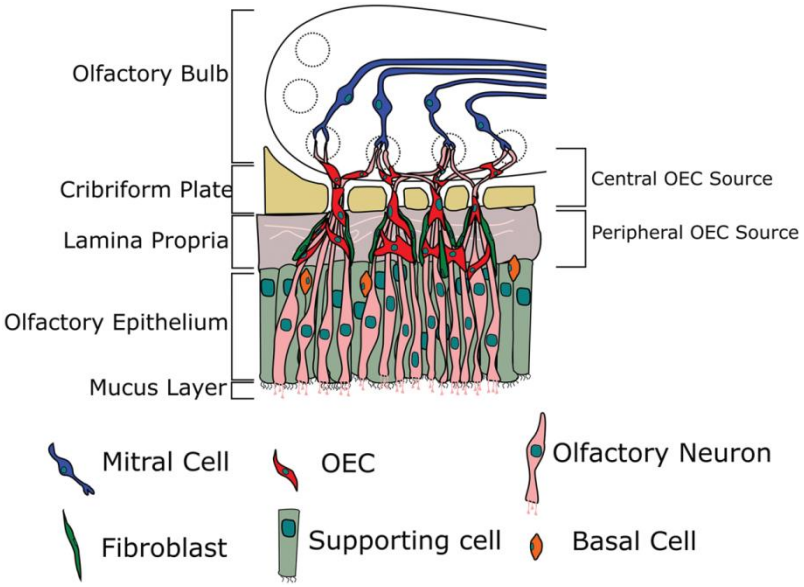

Figure 7. Representation of the ON inside the nasal cavity, medial view. Olfactory neurons located in the ON extend axons from the nasal cavity through the lamina propria and the cribriform plate to reach the olfactory bulb. Image from (Wright et al. 2018).

Throughout adult life, olfactory sensory neurons are regenerated from neuronal precursors (Leung, Coulombe, and Reed 2007; Brann and Firestein 2014), being therefore an accessible source of regenerating neural cells (Borgmann-Winter et al. 2009). The value of the ON relies on its capacity to generate pluripotent cells that can proliferate *in vitro* and differentiate into multiple cell types (Matigian et al. 2010), thus generating a valid surrogate of human CNS tissue (Lavoie et al. 2017).

Changes in gene regulation and/or expression, altered metabolic or molecular processes detected in the ON could mirror abnormal neurodevelopmental processes related to the pathophysiology of certain neuropsychiatric disorders (Smutzer et al. 1998) thus opening a new, very promising window to the study of neuropsychiatric disorders (Borgmann-Winter et al. 2015; Mackay-Sim 2012; Benitez-King et al. 2016), particularly those that may have a possible relationship with neurodevelopmental alterations, such as schizophrenia (Insel 2010).

Interestingly, expression of CB_1R, and the endocannabinoid 2-AG has been found in the ON of experimental animals (Breunig et al. 2010; Hutch et al. 2015). Further, human ON cells exhibit a neuronal phenotype comprising several types of receptors including 5-$HT_{2A}R$, and signaling pathways related to olfaction and other functional aspects of the CNS (Borgmann-Winter et al. 2009)

Early studies involving the ON to study schizophrenia obtained the samples through autopsy and examined distribution of developmentally regulated cytoskeletal proteins, degree of neuronal maturity and integrity of synaptic connections (Smutzer et al. 1998; Arnold et al. 2001; Rioux et al. 2005), but limitations related to the need of premortem consent, rapid degradation of post-mortem tissue, patient's generally old age and small sample sizes obtained over long periods of time, all of which may have limited result generalizability and the hampered the continued use of this technique, that has been progressively replaced by the use of alternative approaches.

Biopsies of the ON have been able to generate valid models and cultures to study genetic and protein expression, cell cycle alterations and proliferation, cell function or intracellular signalling alterations in schizophrenia (English et al. 2015; Fan et al. 2012; Mor et al. 2013; Brown et al. 2014; Borgmann-Winter et al. 2016; Horiuchi et al. 2016; Tee et al. 2017; Igolkina et al. 2018). However, biopsies usually require the involvement of an experienced otorhinolaryngologist, and are either obtained from totally anesthetized subjects during nasal surgery (Marshall et al. 2005) or through the use of nasal endoscopy after injection of local anaesthesia (Gao et al. 2017) and may have variable rates of success potentially requiring abstraction of multiple samples.

More recently, a non-invasive procedure has been described as an alternative to biopsies to obtain viable ON tissue from the nasal cavity (Benítez-King et al. 2011), whereby cells are exfoliated through circular movements and cultured to propitiate neural growth **(Figure 8),** which has further allowed the study of the ON at a larger scale (Muñoz-Estrada et al. 2015; Muñoz-estrada et al. 2018; Idotta et al. 2019). For the work related to this thesis, we have taken advantage of this procedure to characterize CB_1R-5-$HT_{2A}R$ heteromers in the ON of cannabis users and schizophrenia patients.

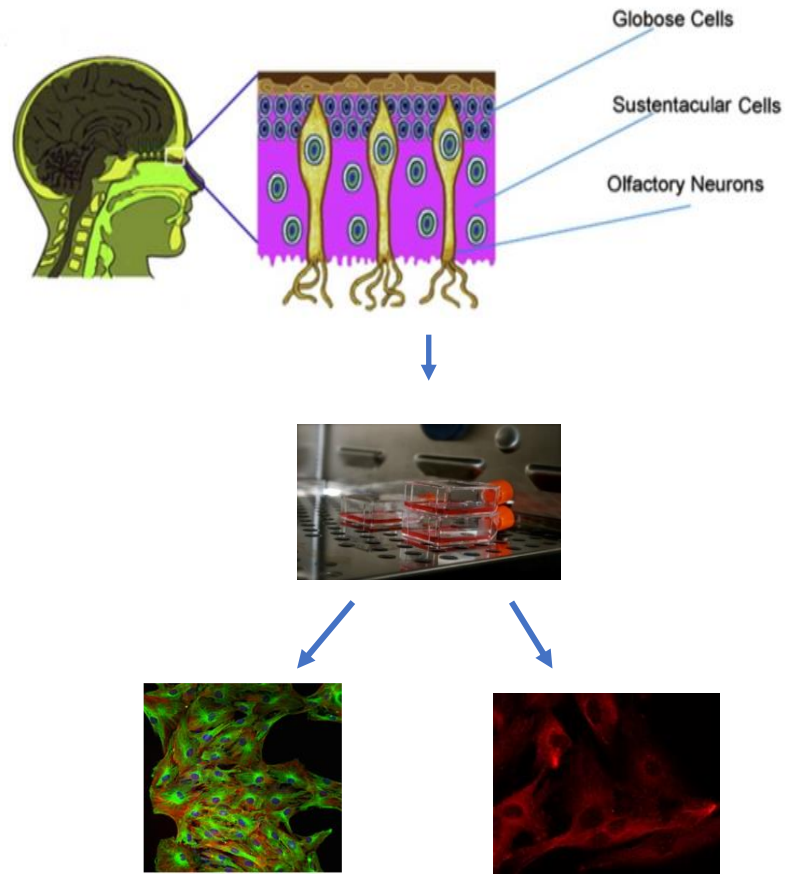

Figure 8. Representation of sample collection and processing. After humidification of the nasal cavity, two separate sterile interdental brushes are used to obtain ON samples, which are subsequently cultured.


THC
CB1R
5-HT2AR

HYPOTHESES AND OBJECTIVES

HYPOTHESES

The first hypothesis of the project is that the olfactory neuroepithelium represents a relevant substrate to explore novel biomarkers related to neuropsychiatric conditions, such as chronic cannabis use and schizophrenia. Second, that cannabis use could induce changes in the formation and the signalling properties of CB_1-5-HT_2A heteromers. Third, the expression and function of this heteromer may also be altered in schizophrenia, and could be modulated by cannabis use, and by antipsychotic treatment. Fourth, that the expression of CB_1-5-HT_2A heteromers could be linked to cognitive performance.

SPECIFIC OBJECTIVES

1. Study the cellular, molecular, and electrophysiological properities of olfactory neuroepithelial cells in healthy control subjects and cannabis users.

2. Determine the expression and functionality of CB_1-5-HT_2A heteromers in olfactory neuroepithelium cells of healthy controls and chronic cannabis users.

3. Evaluate the formation and functionality of CB_1-5-HT_2A heteromers in the olfactory neuroepithelium of schizophrenic patients (cannabis users and non-cannabis users), as compared to healthy controls (cannabis users and non-cannabis users).

4. Correlate the expression of CB_1-5-HT_2A heteromers with features related to the use of cannabis, to cognitive performance, neurological soft signs, and clinical variables.

5. Explore the *in vitro* effects of antipsychotic treatment in olfactory neuroepithelium cells from healthy controls and chronic cannabis users on the functionality of CB_1-5-HT_2A heteromers.


THC
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RESULTS

ARTICLE 1

"Cannabis Users show Enhanced Expression of CB_1-5-HT_2A Receptor Heteromers in Olfactory Neuroepithelium Cells"

Galindo L, Moreno E, López-Armenta F, Guinart D, Cuenca-Royo A, Izquierdo-Serra M, Xicota L, Fernandez C, Menoyo E, Fernández-Fernández JM, Benítez-King G, Canela EI, Casadó V, Pérez V, de la Torre R, Robledo P.

Mol Neurobiol. 2018 Aug;55(8):6347-6361. doi: 10.1007/s12035-017-0833-7

OBJECTIVE

To investigate the expression and functionality of CB_1R-5-HT_2AR heteromers in the olfactory neuroepithelium of chronic cannabis users, and to evaluate their role in cognitive alterations.

ABSTRACT

Cannabinoid CB1 receptors (CB_1R) and serotonergic 2A receptors ($5HT_{2A}R$) form heteromers in the brain of mice where they mediate the cognitive deficits produced by delta9-tetrahydrocannabinol. However, it is still unknown whether the expression of this heterodimer is modulated by chronic cannabis use in humans. In this study, we investigated the expression levels and functionality of CB_1R-$5HT_{2A}R$ heteromers in human olfactory neuroepithelium (ON) cells of cannabis users and control subjects and determined their molecular characteristics through adenylate cyclase and the ERK 1/2 pathway signaling studies. We also assessed whether heteromer expression levels correlated with cannabis consumption and cognitive performance in neuropsychological tests. ON cells from controls and cannabis users expressed neuronal markers such as βIII-tubulin and nestin, displayed similar expression levels of genes related to cellular self-renewal, stem cell differentiation, and generation of neural crest cells, and showed comparable Na^+ currents in patch clamp recordings. Interestingly, CB_1R-$5HT_{2A}R$ heteromer expression was significantly increased in cannabis users and positively correlated with the amount of cannabis consumed, and negatively with age of onset of cannabis use. In addition, a negative correlation was found between heteromer expression levels and attention and working memory performance in cannabis users and control subjects. Our findings suggest that cannabis consumption regulates the formation of CB_1R-$5HT_{2A}R$ heteromers and may have a key role in cognitive processing. These heterodimers could be potential new targets to develop treatment alternatives for cognitive impairments.


THC
CB1R
5-HT2AR

ARTICLE 2

"Altered signalling in CB1R-5-HT2AR heteromers in olfactory neuroepithelium cells of schizophrenia patients is modulated by cannabis use"

Guinart D, Moreno E, Galindo L, Cuenca-Royo A, Barrera-Conde M, Pérez EJ, Fernández-Avilés C, Correll CU, Canela EI, Casadó V, Cordomi A, Pardo L, de la Torre R, Pérez V, Robledo P.

Schizophr Bull. 2020 Apr 6:sbaa038. doi: 10.1093/schbul/sbaa038. Epub ahead of print.

PMID: 322493

OBJECTIVE

To investigate the changes in the expression and functionality of CB1R-5-HT2AR-HET in olfactory neuroepithelial cells in schizophrenia patients, as well as their clinical and neurocognitive correlates and their modulation by cannabis use.

ABSTRACT

Schizophrenia (SCZ) has been associated with serotonergic and endocannabinoid systems dysregulation, but difficulty in obtaining *in vivo* neurological tissue has limited its exploration. We investigated CB_1R-5-$HT_{2A}R$ heteromer expression and functionality via intracellular pERK and cAMP quantification in olfactory neuroepithelium (ON) cells of SCZ patients non-cannabis users (SCZ/nc), and evaluated whether cannabis modulated these parameters in patients using cannabis (SCZ/c). Results were compared versus healthy controls non-cannabis users (HC/nc) and healthy controls cannabis users (HC/c). Further, antipsychotic effects on heteromer signalling were tested *in-vitro* in HC/nc and HC/c. Results indicated that heteromer expression was enhanced in both SCZ groups versus HC/nc. Additionally, pooling all four groups together, heteromer expression correlated with worse attentional performance and more neurological soft signs (NSS), indicating that these changes may be useful markers for neurocognitive impairment. Remarkably, the previously reported signalling properties of CB_1R-5-$HT_{2A}R$ heteromers in ON cells were absent specifically in SCZ/nc treated with clozapine. These findings were mimicked in cells from HC/nc exposed to clozapine, suggesting a major role of this antipsychotic in altering the quaternary structure of the CB_1R-5-$HT_{2A}R$ heteromer in SCZ/nc patients. In contrast, cells from SCZ/c showed enhanced heteromer functionality similar to HC/c. Our data highlight a molecular marker of the interaction between antipsychotic medication and cannabis use in SCZ with relevance for future studies evaluating its association with specific neuropsychiatric alterations.


THC
CB1R
5-HT2AR

DISCUSSION

1. Characterization of ON cells in healthy controls and cannabis users

In this project, we successfully obtained ON samples using a non-invasive exfoliation procedure in living humans. Consistent with several reports in the literature (Jiménez-Vaca et al. 2018; Lavoie, Sawa, and Ishizuka 2017), we found that the ON constitutes a relevant substrate to investigate novel biomarkers related to the pathophysiology of neuropsychiatric diseases, such as chronic cannabis use and schizophrenia.

The advantages of exfoliation over other types of tissue obtention techniques, such as biopsy and autopsy are multiple. First, autopsies are limited by the fact that investigators need premortem consent, and there is a risk of rapid sample degradation, patients tend to be old and sample sizes are small, thus limiting result generalizability. Further, the fact that exfoliation is essentially non-invasive and basically consists of a simple brushing of the nostrils allows for quicker assessments that can be performed by any sanitary personnel with a remarkably high success rate. Thus, sample extraction does not require an otorhinolaryngologist to perform an endoscopy under anaesthesia, as usually done in the case of biopsies that also tend to present a very heterogeneous cellular sample (Benítez-King et al. 2011; Lavoie, Sawa, and Ishizuka 2017). In fact, in this study, the exfoliation procedure was conducted in all subjects without exception, and there were no incidences related to the procedure, aside from occasional discomfort.

However, some additional elements must be considered. In our sample, to minimize discomfort, particularly brushing-related erosion of highly innervated intranasal mucosa, topic lidocaine was applied in the area using a nasal spray shortly before the extraction. The influence that the administration of lidocaine may have had in our findings cannot be dismissed. For instance, a study showed that application of nasal lidocaine spray to the nasal mucosa of healthy volunteers caused a small reduction in olfactory perception (Hari, Grimshaw, and Jacob 2018). It is important to note, though, that this reduction was transient, returning to normal levels after 15-30 minutes, which excludes persistent neural damage, and the doses used were much higher than in our study.

Additional testament to the quality of the exfoliated tissue is that the all the cells tested expressed markers of neural lineage. In the first experiment, including a subset of ON cells exfoliated from healthy controls and cannabis users (ARTICLE 1), cells from both groups were positive for βIII-tubulin and nestin, two specific neuronal markers. βIII-tubulin identifies both mature and immature neurons, whereas nestin identifies neural precursors (Benítez-King et al. 2011). Both βIII-tubulin and nestin have been used as neural and neural precursor markers in multiple previous studies (Hill et al. 2019; Ortiz-López et al. 2017; Benítez-King et al. 2011). Further, among these two groups of subjects, we explored differences in terms of βIII-tubulin and nestin protein levels, both by immunoblot and immunofluorescence techniques, and we found no significant group differences, confirming an equivalent stage of cell differentiation in both groups.

In addition, we also compared cannabis users and healthy controls in terms of the expression of genes related to cell differentiation and cell function. In both groups, we found expression of genes associated with stem cell differentiation, self-renewal, and generation of neural crest cell (namely: *NANOG, TWIST, RET* and *P63*), further corroborating the neural lineage of the ON cells in culture. No changes in the relative expression of these genes were found between groups. On the other hand, we did not find expression of characteristic molecular markers of neuronal progenitors, globose or horizontal basal cells (*PAX6*, *SOX2*, and *NeuroD1*), or markers of olfactory ensheathing cells (*P75*) in either group (Borgmann-Winter et al. 2015)

Furthermore, the electrophysiological recordings in ON samples from cannabis users and control subjects revealed the presence of voltage-gated Na^+ channels that exhibited electrophysiological properties consistent with previous data (Benítez-King et al. 2011), but were not different between cells derived from cannabis users and control subjects. Taken together, these data indicate that the ON cell cultures obtained in our studies were of neural lineage at various stages of neuronal differentiation.

Further, these data also indicate that cannabis use does not induce mayor perturbations in ON cell viability, which guarantees the effective analysis of functional biomarkers of cannabis use disorder in this cell model. Our data, identifying several cellular, functional and gene expression aspects of human ON samples, highlight their potential for the non-invasive, inexpensive detection of disease or prognostic biomarkers (Idotta et al. 2019), This can be relevant even preceding clinical symptom onset, as it is interestingly common for olfactory alterations to be present before neuropsychiatric alterations appear, such as in the case of schizophrenia (Radua et al. 2018) or Alzheimer's disease (Jung, Shin, and Lee 2019), but also as early marker of illness progression, such as in the case of multiple sclerosis (Ciurleo et al. 2018).

2. Cognitive performance and clinical implications in cannabis users and schizophrenia patients

We report significant findings related to cognitive performance of our sample. First, our results show that cannabis users showed attentional and working memory deficits compared to healthy control non-cannabis users (ARTICLE 1 and 2). Our findings are aligned with multiple previous studies relating cannabis use to psychomotor, mood and cognitive alterations (Mizrahi, Watts, and Tseng 2017; D'Souza et al. 2004; Volkow et al. 2016), including altered neural function during attention and working memory (Weinstein, Livny, and Weizman 2016).

Additionally, we found that schizophrenia subjects who do not use cannabis showed widespread cognitive deficits: attention, working memory, executive functions and emotional recognition compared to healthy controls and cannabis users (ARTICLE 2). These findings were replicated in schizophrenia patients who use cannabis, except for working memory, which seems to be spared in this subset of subjects. Thus, in our sample, schizophrenia patients who do not use cannabis were more cognitively compromised than schizophrenia patients who use cannabis.

Cognitive deficits are considered a central feature of schizophrenia and range across a number of cognitive domains, including attention, verbal fluency, working memory, and processing speed, with superimposed severe deficits in declarative verbal memory and executive functioning (Fatouros-Bergman et al. 2014; Bowie and Harvey 2006). Our results are consistent with these reports. Current pharmacological approaches either with antipsychotics or with other types of drugs have shown little impact on improving cognition in schizophrenia (Choi, Wykes, and Kurtz 2013; Harvey 2009), highlighting the need for unveiling new molecular mechanisms and developing new treatment approaches. Therefore, the characterization of novel molecular targets that may play a role in cognitive alterations frequently seen in schizophrenia is of high interest of the field.

Interestingly, in line with our results, some studies describe better cognitive profile in schizophrenia patients using cannabis compared to schizophrenia patients who do not use cannabis (Yucel et al. 2012). While some authors have interpreted these results as a bias derived from a greater capacity for socialization, and therefore to obtain cannabis for consumption in patients with better functioning (Potvin and Amar 2008) others interpret that the schizophrenia group with cannabis consumption could be a subgroup of patients with less congenital vulnerability (such as less neurocognitive symptoms and less presence of NSS (Yucel et al. 2012; Ruiz-Veguilla, Callado, and Ferrin 2012) where environmental factors, such as use of cannabis, would play a more relevant role in precipitating illness onset.

Previous studies have correlated cellular and molecular changes in ON cells with cognive alterations in schizophrenia patients. One study found that cell proliferation was altered in exfolliated ON samples from schizophrenia patients compared to healthy controls, and such changes correlated with verbal memory alterations (Idotta et al. 2019). However, sample size was small, did not account for drug use, and all patients had been hospitalized recently, which had probably led to recent medication changes the authors cannot account for.

Another study, using nasal biopsies and laser capture microdissection in a sample of 16 schizophrenia patients and 15 healthy controls, correlated neuropsychological domain scores with differential gene expression in the ON related to the SMAD pathway (Horiuchi et al. 2016). The authors found that two genes, the SMAD family member 5 (*SMAD5*), and the poly(A) binding protein, cytoplasmic 4-like (*PABPC4L*), were positively correlated to the overall composite score of the neuropsychological assessment. These findings confirm the utility of the ON to study molecular markers of disease with clinical significance.

3. Increased CB_1R-5-HT_2AR heteromer expression in ON cells of cannabis users and schizophrenia patients

In this project, we revealed the presence of functional CB_1R-5-HT_2AR heteromers in ON cells of healthy controls (cannabis users and non-cannabis users), and schizophrenia patients (cannabis users and non-cannabis users) (ARTICLES 1 and 2).

Notably, CB_1R-5-HT_2AR heteromer expression was increased in cannabis users and in schizophrenia patients, as compared to healthy controls non-cannabis users. Our findings are aligned with previous studies in animal models reporting that acute THC administration increased the number of CB_1R-5-$HT_{2A}R$ heteromers in the brain of mice (Vinals et al. 2015). In addition, the capacity of cannabis to induce the formation of other types of heteromers in neural tissue has been described. Thus, chronic THC increased the number of dopamine D_1R-D_2R heteromers in striatal neurons of monkeys (Hasbi et al. 2020). Further, other authors recently showed that cannabidiol blunts THC-induced cognitive impairment in rodents via $A_{2A}R$-CB_1R heteromers expressed at the presynaptic level in hippocampal neurons (Aso et al. 2019).

Interestingly, we found a significant positive correlation between the expression of CB_1R-$5HT_{2A}R$ heteromers and the amount of cannabis consumption in cannabis users (as measured by plasma concentrations of THC-COOH) (ARTICLE 1), which remained significant when including schizophrenia patients that used cannabis (ARTICLE 2).

Although it was not possible to characterize in detail the type, potency or exact quantity of cannabis use, we did measure levels of metabolite THC-COOH as a valid proxy which can be detected between 2-7 days after use (Huestis, Henningfield, and Cone 1992). Moreover, in the population of subjects that had tried cannabis at least once in their lifetime, we found a significant positive correlation between the age of first onset of cannabis use and heteromer expression. Hence, the use of cannabis seemed to favor heteromer formation in both healthy controls and schizophrenia patients.

Importantly, CB_1R-5-HT_2AR heteromer expression in schizophrenia patients was increased independently of cannabis use. These findings suggest that cannabis stabilizes the formation of this heteromer in healthy controls, but that in schizophrenia patients, the development and/or treatment with antipsychotics may preclude the potential effects of cannabis consumption. Therefore, we cannot completely exclude the possibility that a combination of epigenetic factors taking place in this population may be modulating the expression of CB_1R-5-HT_2AR heteromers. In this sense, recent studies have identified gene-environment interactions between a genetic polymorphism in the endocannabinoid system and cannabis use involved in the risk of presenting a psychotic episode (Bioque et al. 2019), and epigenetic changes have been shown to take place in schizophrenia patients (Roth et al. 2009; Wockner et al. 2014).

4. CB_1R-5-HT_2AR heteromer expression is linked to attention deficits and NSS

A major finding of this study was that enhanced heteromer formation correlated with worse attention performance, and with more NSS in the entire population studied.

The presence and severity of NSS does not relate to a specifically located neuronal pathology, but rather is indicative of a nonspecific impairment either in sensory-motor integration or in cortical-subcortical connections (Gunasekaran, Venkatesh, and Asokan 2016). Importantly, these functions are modulated in part by corticostriatal circuits that highly express CB_1R (Fernandez-Ruiz 2009; Goodman and Packard 2015).

Therefore, our results showing a possible link between CB_1R-5-$HT_{2A}R$ heteromer expression in ON cells and NSS-related processes in the CNS is neuroanatomically plausible. Longitudinal studies would be needed to further understand this relationship and would help to clarify whether CB_1R-5-$HT_{2A}R$ expression maintains a correlation with NSS overtime.

Attention refers to the capacity to maintain voluntary focus on a single task, and it is a critical cognitive function that allows individuals to effectively interact with their environments and complete goals (Fortenbaugh et al. 2018). Neuroanatomical or molecular correlates of attention have not yet been clearly elucidated, partly because of the complexity to isolate attention from other simultaneous cognitive processes. Areas of the prefrontal and parietal cortices have been traditionally implicated in tasks requiring the deployment and maintenance of attention (Langner and Eickhoff 2013). However, more recently, some attention-related functional brain networks have been identified, seemingly emerging from coordinated activity across wide swaths of cortex as well as subcortical regions and the cerebellum, adding to the difficulty of underpinning the underlying molecular correlates of attention (Katsuki and Constantinidis 2014; Rosenberg et al. 2016).

Interestingly, as outlined in the introduction, both CB_1R and 5-$HT_{2A}R$ are widely expressed across both cortical and subcortical regions with a relatively high density compared to other receptors from the same neurotransmitter family, and recent findings show that stress-related serotonin hypersecretion originating in the dorsal raphe nucleus could cause disruption in the activity of cortical neurons in the anterior cingulate and dorsolateral frontal cortices (Eggers 2013). Further, recent studies in primates show that enhancing central serotonergic function results in categorically distinct changes in fundamental cognitive operations such as attention (Weinberg-Wolf et al. 2018). Thus, it is possible that increased formation of CB_1R-5$HT_{2A}R$ heteromers in ON cells may biologically link to neurocognitive impairments related to attentional performance, as well as non-specific motor coordination and sensorimotor integration alterations.

Conversely, no significant correlations were observed regarding other neurocognitive measures, such as working memory, verbal fluency or emotional recognition, as well as for severity of psychotic or depressive symptoms in schizophrenia patients, suggesting an apparent dissociation between heteromer expression and these processes. However, we must consider that fact that both groups of patients were treated outpatients that showed a relatively mild symptoms profile, with median PANSS positive and Hamilton Depression Scale scores moderately to very low, which may have prevented us from detecting a meaningful relationship with CB_1R-$5HT_{2A}R$ expression.

Additionally, is also possible that our modest sample size may have hampered our chances of finding meaningful relationships and thus limiting the generalizability of the results. However, the group with the smallest sample size (schizophrenia patients that use cannabis) tended to have a very tight interquartile range along all tests, thus making statistical inferences more reliable. While patient recruitment and obtention of ON samples may be challenging in inpatient settings, studies with more severe patients both in terms of depressive and psychotic symptoms would be needed to further study the relationship between CB_1R-$5HT_{2A}R$ heteromers and psychotic and/or depressive symptoms.

Another element we must consider is the fact that all patients were treated with a variety of antipsychotics, but also other medications, including antidepressants. Some recent naturalistic studies report an association between lifetime antipsychotic dose and poorer global cognition in a general population cohort (Husa et al. 2017). Also, high dopamine D_2R occupancy, which is a common mechanism of action of antipsychotic drugs, has been associated with increased risk of cognitive impairment (Sakurai et al. 2013). However, other studies (Davidson et al. 2009; Keefe et al. 2007) have reported cognitive improvements, particularly in the early stages of the illness, although improvements were modest and differences between antipsychotic classes were not detected. In contrast, a recent meta-analysis seems to suggest an overall positive effect of antipsychotics on cognition, with an apparently small advantage of atypical antipsychotics versus typical antipsychotics (Desamericq et al. 2014).

Finally, in our study we did not evaluate olfactory performance; therefore we cannot rule out completely that CB_1R-5-$HT_{2A}R$ heteromers in ON cells could be linked to olfactory alterations obsereved in schizophrenia patients (Nguyen, Shenton, and Levitt 2010), and that cannabis use may modulate this interaction. Indeed, endocannabinoids and exogenous cannabinoids increase odor detection and food intake by acting on CB_1R located in the olfactory bulb in mice (Soria-Gomez, Bellocchio, and Marsicano 2014), although contrasting results have also been reported (Hutch et al. 2015) and there is a dense serotonergic innervation of the olfactory bulb in mammals, where 5-$HT_{2A}R$ could participate in olfactory learning (Hardy et al. 2005). Future studies may help to further understand the potential involvement of CB_1R-5-$HT_{2A}R$ heteromers in ON cells and/or the olfactory bulb in odor detection and disease.

5. CB_1R-5-$HT_{2A}R$ heteromer signalling is altered in ON cells of cannabis users and schizophrenia patients

To determine changes in the functionality of CB_1R-5-$HT_{2A}R$ heteromers, we assessed cAMP production and pERK activity in ON cells following pharmacological challenge with different agonists and antagonists, and evaluated crosstalk and cross-antagonism via the heteromer (ARTICLE 1 and 2). Our results revealed that the negative crosstalk and cross-antagonism was more intense in cannabis users as compared to healthy controls, consistent with previous findings in mice showing that acute administration of THC enhances the functionality of the heteromer (Vinals et al. 2015).

In contrast, we found that schizophrenia patients that do not use cannabis did not display negative crosstalk and or cross-antagonism with respect to all the other groups, while in schizophrenia patients that use cannabis, both of these effects were present. These data suggested that (i) schizophrenia and/or antipsychotic treatment was potentially changing the conformation of the heteromer leading to the loss of the known molecular signature of the heteromer, and (ii) that cannabis (THC) prevents this alteration.

To further understand these data, we designed an *in vitro* study where ON cells from healthy controls with no previous history of cannabis use, and ON cells from cannabis users were treated repeatedly with several different types of antipsychotics, including clozapine (high affinity 5-HT_{2A}R antagonist), aripiprazole (moderate affinity 5-HT_{2A}R antagonist, but also D_2R partial agonist), and haloperidol (low affinity 5-HT_{2A}R antagonist, preferential D_2R antagonist).

Our results showed that in ON cells from healthy controls with no cannabis use, heteromer functionality was lost (no negative crosstalk or cross-antagonism) following clozapine, but not aripiprazole or haloperidol administration. These findings were corroborated when we analyzed the functionality of the heteromer in ON cells from separate sub-groups of schizophrenia patients treated with either clozapine/olanzapine or aripiprazole/risperidone.

In contrast, in ON cells from cannabis users treated with clozapine, aripiprazole and haloperidol, heteromer functionaliy was enhanced (more negative crosstalk and cross-antagonism). The implication of these results is that clozapine, but not aripiprazole nor haloperidol, binds to 5-HT_{2A}R in the heteromer in a way that changes its conformation, and that cannabis use (THC) prevents this alteration.

Our docking experiments shed light into the possible mechanisms taking place in the heteromer that could explain this change in conformation. Thus, we proposed that clozapine binding at 5-HT_{2A}R in schizophrenia patients that do no use cannabis is producing an alternative interface that tolerates simultaneous activation of both receptors (absence of negative cross-talk), and does not contain the cross-antagonism signature. This is possible because in this slightly different quaternary structure of the CB_1R-5-HT_{2A}R heteromer, the outward movement of TM6 in both protomers is feasible, and the lack of formation of the four-helix bundle facilitates receptor activation of the protomer.

Interestingly, when THC was docked in these simulations, we observed that it triggered an extra stabilization of the TMs 5&6 interface in a way that clozapine is no longer able to produce the alternative interface. A possible explanation for such findings would be that exposure to clozapine implies the participation of additional proteins in the heteromer that stabilize the alternative interface, and that cannbis use precludes this interaction. Further studies would be required to validate this model and identify the components of these larger heteromeric complexes.

6. Implications of CB_1R-5-$HT_{2A}R$ heteromer signalling alterations

The changes in heteromer functionality observed are difficult to correlate with neuropsychological and clinical performance. In this study, we did not have enough sample size to compare clinical or cognitive variables in schizophrenia patients based on their underlying antipsychotic treatment, which would have sliced our sample too thin.

Nonetheless, our results suggest that both potentiation of heteromer functionality due to cannabis use, and loss of its known molecular signature in clozapine-treated schizophrenia patients may lead to cognitive alterations. Also, our findings show that cannabis counteracts the effects of clozapine on heteromer functionality, but whether this interaction is detrimental or beneficial for cognitive performance or other aspects, still needs to be elucidated.

In relation to the effects of clozapine vs. other antipsychotics on cognitive performance, some studies show protective effects (Meltzer and McGurk 1999; Lee, Jayathilake, and Meltzer 1999), while others report dose-dependent detriments (Savulich et al. 2018) or no differences vs. other atypical antipsychotics (Asenjo Lobos et al. 2010; Bilder et al. 2002). While in these studies the influence of cannabis was not specifically accounted for; several other reports exist regarding the effects of clozapine in schizophrenia patients with comorbid cannabis use.

A recent retrospective cohort study showed that both cannabis use and psychotic symptoms decreased in patients treated with clozapine, compared to patients treated with other antipsychotics in adolescents with psychosis (Tang, Ansarian, and Courtney 2017). Other retrospective chart review studies have shown similar outcomes in adults (Green et al. 2003).

Interestingly, quite a few studies have evaluated the differential effects of antipsychotics in reducing cannabis consumption in schizophrenia patients. Thus, a small clinical trial randomizing 31 patients to clozapine vs. continuing other antipsychotic treatment, found that use of cannabis was reduced in the clozapine group, but not significantly (Brunette et al. 2011). Sample size and lack of power may have hampered their ability to find significantly different results. However, other randomized clinical trials comparing clozapine to ziprasidone have found no differences (Schnell et al. 2014), but clozapine dose was low.

While a reduction in cannabis use could be mediated by some improvements in cognition and impulse control related to clozapine use (Meltzer and McGurk 1999; Dursun et al. 2000), it is also possible that clozapine is able to reduce cannabis use simply via improvement of psychotic symptoms. Thus, if patients are using cannabis to cope with psychotic symptoms or to self-medicate (Mané et al. 2015; Leweke et al. 2012), a potentially more effective antipsychotic (clozapine) may essentially reduce/eliminate the need to use cannabis. Also, clozapine's complex pharmacological profile may involve changes in the dopaminergic mesocorticolimbic system, as well as other neurotransmitter systems involved in substance use disorders (Green et al. 2008). Lastly, other confounders could account for such findings, including the fact that treatment with clozapine involves a more intense medical monitoring, and thus more interactions with the clinical team and associated psychosocial services.

Indeed, the need for clinical trials aimed to untangle the differential effects and molecular underpinnings of different antipsychotics on cognition and their interaction with cannabis use is pressing. However, due to the multiple confounders and biases influencing the choice of one antipsychotic versus another, only prospective, randomized clinical trials, where treatment is assigned as part of the study accounting for baseline between-group differences, including cannabis use, would truly help tackle down this complex interaction involving multiple players. Of note, these trials could also involve newer antipsychotics with different pharmacological profiles (Koblan et al. 2020), but also other clozapine-like drugs that may interact with the heteromer in a similar way changing its conformation and disrupting its signaling.

Taken together, our data put forward that the CB_1R-5-$HT_{2A}R$ heteromer could be a molecular marker of the interaction between antipsychotic medication and cannabis use in schizophrenia with relevance for neurocognitive performance. Nonetheless, our design was cross-sectional, so it is unknown if results may change over time. Further research is warranted to understand the potentially evolving role of CB_1R-5-$HT_{2A}R$ heterodimers in the cognitive deficits observed in schizophrenia patients, including the study of antipsychotic naïve schizophrenia population and longitudinal, prospective data. Further, we do not know whether these findings are specific to schizophrenia or may be shared with other neuropsychiatric disorders. Other neuropsychiatric control groups would have been needed to elucidate the diagnostic specificity of our findings.

7. Summary of the findings and concluding remarks

In summary, our results show that cannabis use stabilizes the formation of CB1R-5-$HT_{2A}R$ heteromers in ON cells, and that younger age of onset of cannabis use, and plasma concentrations of THC-COOH are correlated with the expression of the heteromer in both schizophrenia patients and healthy controls who use cannabis.

Further, we show that the formation of CB_1R-5-$HT_{2A}R$ heteromers in ON cells may be detrimental for cognitive processes such as attention and for NSS. Since these results were observed in the entire sample studied, and heteromer expression is increased in healthy cannabis users to the same extent as in schizophrenia patients, it is possible that both cannabis use and schizophrenia stabilize the heteromer leading to the aforementioned neurocognitive alterations. However, the influence of antipsychotic treatment, polytherapy in some cases, cannot be dismissed, and subjects free of antipsychotic treatment will have to be tested to tease out the specific effects of cannabis in CB_1R-5-$HT_{2A}R$ heteromer formation and functionality in schizophrenia.

In addition, our results also show that clozapine, but not aripiprazole or haloperidol, abolishes the characteristic signaling properties of CB_1R-5-$HT_{2A}R$ heteromers in schizophrenia patients. This was confirmed not only in *in vitro* studies but also when separating schizophrenia patients by treatment regimen. Some molecular and pharmacological features may account for this effect.

On the one hand, these data could imply antipsychotics with a large, bulky molecular structure such as clozapine may interact with the 5-$HT_{2A}R$ in the heteromer in a way that changes its conformation and disrups its signaling, while antipsychotics with an elongated, lighter conformation such as aripiprazole or haloperidol do not. On the other hand, an alternative explanation for such findings could lie on the different affinity both drugs display for 5-$HT_{2A}R$. Thus, clozapine's affinity for 5-$HT_{2A}R$ is significantly higher (Correll 2010), and aripiprazole may not bind as preferentially to 5-$HT_{2A}R$, also when forming heteromers such as CB_1R-5-$HT_{2A}R$. Interestingly, cannabis use prevents clozapine's alteration of the quaternary structure of the heteromer, stabilizing its formation, and thus preempts the effect of clozapine in altering the signaling properties of CB_1R-5-$HT_{2A}R$ heteromers.

In conclusion, in this thesis we show that both cannabis and schizophrenia stabilize the formation of CB_1R-5-$HT_{2A}R$ heteromers, but cannabis use and clozapine treatment in schizophrenia patients modify de signaling properties of CB_1R-5-$HT_{2A}R$ in specific yet opposite ways. Finally, we also show that the CB_1R-5-$HT_{2A}R$ heteromer is a potentially relevant biomarker of neurocognitive alterations in a population consisting of healthy controls, cannabis users and schizophrenia patients (cannabis and non-cannabis users).


THC
CB1R
5-HT2AR

CONCLUSIONS

CONCLUSIONS

The findings obtained in the present thesis revealed that:

1. Olfactory neuroepithelial cells of healthy control subjects and cannabis users express proteins associated with neuronal markers, and genes related to neuronal precursors.

2. The olfactory neuroepithelium of healthy control subjects and cannabis users show similar cell differentiation and sodium channel functionality.

3. Functional CB_1R-5-$HT_{2A}R$ heteromers are expressed in the ON of healthy controls, cannabis users and schizophrenia patients.

4. CB_1R-5-$HT_{2A}R$ heteromer formation is increased in cannabis users and schizophrenia patients, with and without a history of chronic cannabis use.

5. A significant link exists between cannabis use and the formation of CB_1R-5-$HT_{2A}R$ heteromers.

6. A significant correlation occurs in the general population between the expression of CB_1R-5-$HT_{2A}R$ heteromers and attention and NSS.

7. The molecular signature of CB_1R-5-$HT_{2A}R$ heteromers is altered in schizophrenia patients, and this effect is prevented by cannabis use.

8. Chronic administration of clozapine *in vitro* to ON cells from healthy controls modifies the functionality of CB_1R-5-$HT_{2A}R$ heteromers, and these changes are not observed in ON cells from cannabis users.

9. Clozapine, but not aripiprazole treatment in schizophrenia patients may induce alterations in the functionality of CB_1R-5$HT_{2A}R$ heteromers through changes in the quaternary structure of the heteromer, and cannabis use prevents these changes.


THC
CB1R
5-HT2AR

REFERENCES

Abdullaev, Yalchin, Michael I Posner, Ray Nunnally, and Thomas J Dishion. 2010. "Functional MRI Evidence for Inefficient Attentional Control in Adolescent Chronic Cannabis Abuse." *Behavioural Brain Research* 215 (1): 45–57. https://doi.org/10.1016/j.bbr.2010.06.023.

Allen, Daniel N, Gerald Goldstein, and Erin Warnick. 2003. "A Consideration of Neuropsychologically Normal Schizophrenia." *Journal of the International Neuropsychological Society : JINS* 9 (1): 56–63. https://doi.org/10.1017/s135561770391006x.

American Psychiatric Association. 2013. *DSM-5: Diagnostic and Statistical Manual of Mental Disorders*. (5th Ed.). Washington, DC.

Arnold, Steven E., Li Ying Han, Paul J. Moberg, Bruce I. Turetsky, Raquel E. Gur, John Q. Trojanowski, and Chang Gyu Hahn. 2001. "Dysregulation of Olfactory Receptor Neuron Lineage in Schizophrenia." *Archives of General Psychiatry* 58 (9): 829–35. https://doi.org/10.1001/archpsyc.58.9.829.

Asenjo Lobos, Claudia, Katja Komossa, Christine Rummel-Kluge, Heike Hunger, Franziska Schmid, Sandra Schwarz, and Stefan Leucht. 2010. "Clozapine versus Other Atypical Antipsychotics for Schizophrenia." *The Cochrane Database of Systematic Reviews*, no. 11 (November): CD006633. https://doi.org/10.1002/14651858.CD006633.pub2.

Aso, Ester, Víctor Fernández-Dueñas, Marc López-Cano, Jaume Taura, Masahiko Watanabe, Isidre Ferrer, Rafael Luján, and Francisco Ciruela. 2019. "Adenosine A(2A)-Cannabinoid CB(1) Receptor Heteromers in the Hippocampus: Cannabidiol Blunts Δ(9)-Tetrahydrocannabinol-Induced Cognitive Impairment." *Molecular Neurobiology* 56 (8): 5382–91. https://doi.org/10.1007/s12035-018-1456-3.

Azofeifa, Alejandro, Margaret E Mattson, Gillian Schauer, Tim McAfee, Althea Grant, and Rob Lyerla. 2016. "National Estimates of Marijuana Use and Related Indicators - National Survey on Drug Use and Health, United States, 2002-2014." *MMWR Surveillance Summaries* 65 (11): 1–25. https://doi.org/10.15585/mmwr.ss6511a1.

Bachmann, Silke, and Johannes Schroder. 2018. "Neurological Soft Signs in Schizophrenia: An Update on the State- versus Trait-Perspective.". *Frontiers in Psychiatry* 8: 272. https://doi.org/10.3389/fpsyt.2017.00272.

Batalla, Albert, Sagnik Bhattacharyya, Murat Yücel, Paolo Fusar-Poli, Jose Alexandre Crippa, Santiago Nogué, Marta Torrens, Jesús Pujol, Magí Farré, and Rocio Martin-Santos. 2013. "Structural and Functional Imaging Studies in Chronic Cannabis Users: A Systematic Review of Adolescent and Adult Findings." *PloS One* 8 (2): e55821. https://doi.org/10.1371/journal.pone.0055821.

Beliveau, Vincent, Melanie Ganz, Ling Feng, Brice Ozenne, Liselotte Højgaard, Patrick M Fisher, Claus Svarer, Douglas N Greve, and Gitte M Knudsen. 2017. "A High-Resolution In Vivo Atlas of the Human Brain's Serotonin System." *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* 37 (1): 120–28. https://doi.org/10.1523/JNEUROSCI.2830-16.2016.

Benítez-King, G, A Riquelme, L Ortíz-López, C Berlanga, M S Rodríguez-Verdugo, F Romo, E Calixto, et al. 2011. "A Non-Invasive Method to Isolate the Neuronal Linage from the Nasal Epithelium from Schizophrenic and Bipolar Diseases." *Journal of Neuroscience Methods* 201 (1): 35–45.

https://doi.org/10.1016/j.jneumeth.2011.07.009.

Benitez-King, G, M Valdes-Tovar, C Trueta, T Galvan-Arrieta, J Argueta, S Alarcon, A Lora-Castellanos, and H Solis-Chagoyan. 2016. "The Microtubular Cytoskeleton of Olfactory Neurons Derived from Patients with Schizophrenia or with Bipolar Disorder: Implications for Biomarker Characterization, Neuronal Physiology and Pharmacological Screening." *Molecular and Cellular Neurosciences* 73 (June): 84–95. https://doi.org/10.1016/j.mcn.2016.01.013.

Bennett, M R. 2011. "Schizophrenia: Susceptibility Genes, Dendritic-Spine Pathology and Gray Matter Loss." *Progress in Neurobiology* 95 (3): 275–300. https://doi.org/10.1016/j.pneurobio.2011.08.003.

Bergé, Daniel, Anna Mané, Purificacion Salgado, Romina Cortizo, Carolina Garnier, Laura Gomez, Cristobal Diez-Aja, Antoni Bulbena, and Victor Pérez. 2016. "Predictors of Relapse and Functioning in First-Episode Psychosis: A Two-Year Follow-Up Study." *Psychiatric Services (Washington, D.C.)* 67 (2): 227–33. https://doi.org/10.1176/appi.ps.201400316.

Bilder, Robert M, Robert S Goldman, Jan Volavka, Pal Czobor, Matthew Hoptman, Brian Sheitman, Jean-Pierre Lindenmayer, et al. 2002. "Neurocognitive Effects of Clozapine, Olanzapine, Risperidone, and Haloperidol in Patients with Chronic Schizophrenia or Schizoaffective Disorder." *The American Journal of Psychiatry* 159 (6): 1018–28. https://doi.org/10.1176/appi.ajp.159.6.1018.

Bioque, Miquel, Bibiana Cabrera, Borja García-Bueno, Karina S Mac-Dowell, Carla Torrent, Pilar A Saiz, Mara Parellada, et al. 2016. "Dysregulated Peripheral Endocannabinoid System Signaling Is Associated with Cognitive Deficits in First-Episode Psychosis." *Journal of Psychiatric Research* 75 (April): 14–21. https://doi.org/10.1016/j.jpsychires.2016.01.002.

Bioque, Miquel, Sergi Mas, Maria Cristina Costanzo, Bibiana Cabrera, Antonio Lobo, Ana Gonzalez-Pinto, Elisa Rodriguez-Toscano, et al. 2019. "Gene-Environment Interaction between an Endocannabinoid System Genetic Polymorphism and Cannabis Use in First Episode of Psychosis." *European Neuropsychopharmacology : The Journal of the European College of Neuropsychopharmacology* 29 (6): 786–94. https://doi.org/10.1016/j.euroneuro.2019.04.005.

Bitter, I, P Czobor, A Borsi, L Fehér, B Z Nagy, M Bacskai, P Rakonczai, et al. 2017. "Mortality and the Relationship of Somatic Comorbidities to Mortality in Schizophrenia. A Nationwide Matched-Cohort Study." *European Psychiatry : The Journal of the Association of European Psychiatrists* 45 (September): 97–103. https://doi.org/10.1016/j.eurpsy.2017.05.022.

Björk Brämberg, Elisabeth, Jarl Torgerson, Anna Norman Kjellström, Peder Welin, and Marie Rusner. 2018. "Access to Primary and Specialized Somatic Health Care for Persons with Severe Mental Illness: A Qualitative Study of Perceived Barriers and Facilitators in Swedish Health Care." *BMC Family Practice* 19 (1): 12. https://doi.org/10.1186/s12875-017-0687-0.

Bloomfield, Michael A P, Chandni Hindocha, Sebastian F Green, Matthew B Wall, Rachel Lees, Katherine Petrilli, Harry Costello, M Olabisi Ogunbiyi, Matthijs G Bossong, and Tom P Freeman. 2019. "The Neuropsychopharmacology of Cannabis: A Review of Human Imaging Studies." *Pharmacology & Therapeutics* 195 (March): 132–61. https://doi.org/10.1016/j.pharmthera.2018.10.006.

Bobes, Julio, Celso Arango, Margarida Garcia-Garcia, and Javier Rejas. 2010. "Prevalence of Negative

Symptoms in Outpatients with Schizophrenia Spectrum Disorders Treated with Antipsychotics in Routine Clinical Practice: Findings from the CLAMORS Study." *The Journal of Clinical Psychiatry* 71 (3): 280–86. https://doi.org/10.4088/JCP.08m04250yel.

Bombin, Igor, Celso Arango, and Robert W Buchanan. 2005. "Significance and Meaning of Neurological Signs in Schizophrenia: Two Decades Later." *Schizophrenia Bulletin* 31 (4): 962–77. https://doi.org/10.1093/schbul/sbi028.

Borgmann-Winter, Karin E, N E Rawson, Hoau Yan Wang, H Wang, M L Macdonald, M H Ozdener, K K Yee, et al. 2009. "Human Olfactory Epithelial Cells Generated in Vitro Express Diverse Neuronal Characteristics." *Neuroscience* 158 (2): 642–53. https://doi.org/10.1016/j.neuroscience.2008.09.059.

Borgmann-Winter, Karin E, S L Willard, D Sinclair, N Mirza, B Turetsky, S Berretta, and C-G Hahn. 2015. "Translational Potential of Olfactory Mucosa for the Study of Neuropsychiatric Illness." *Translational Psychiatry* 5 (March): e527. https://doi.org/10.1038/tp.2014.141.

Borgmann-Winter, Karin E., Hoau Yan Wang, Rabindranath Ray, Brooke R. Willis, Paul J. Moberg, Nancy E. Rawson, Raquel E. Gur, Bruce I. Turetsky, and Chang Gyu Hahn. 2016. "Altered g Protein Coupling in Olfactory Neuroepithelial Cells from Patients with Schizophrenia." *Schizophrenia Bulletin* 42 (2): 377–85. https://doi.org/10.1093/schbul/sbv129.

Borroto-Escuela, Dasiel O, Jens Carlsson, Patricia Ambrogini, Manuel Narváez, Karolina Wydra, Alexander O Tarakanov, Xiang Li, et al. 2017. "Understanding the Role of GPCR Heteroreceptor Complexes in Modulating the Brain Networks in Health and Disease." *Frontiers in Cellular Neuroscience* 11: 37. https://doi.org/10.3389/fncel.2017.00037.

Bowie, Christopher R, and Philip D Harvey. 2006. "Treatment of Cognitive Deficits in Schizophrenia." *Current Opinion in Investigational Drugs (London, England : 2000)* 7 (7): 608–13.

Bowie, Christopher R, Winnie W Leung, Abraham Reichenberg, Margaret M McClure, Thomas L Patterson, Robert K Heaton, and Philip D Harvey. 2008. "Predicting Schizophrenia Patients' Real-World Behavior with Specific Neuropsychological and Functional Capacity Measures." *Biological Psychiatry* 63 (5): 505–11. https://doi.org/10.1016/j.biopsych.2007.05.022.

Brann, Jessica H, and Stuart J Firestein. 2014. "A Lifetime of Neurogenesis in the Olfactory System." *Frontiers in Neuroscience* 8: 182. https://doi.org/10.3389/fnins.2014.00182.

Breunig, Esther, Ivan Manzini, Fabiana Piscitelli, Benjamin Gutermann, Vincenzo Di Marzo, Detlev Schild, and Dirk Czesnik. 2010. "The Endocannabinoid 2-Arachidonoyl-Glycerol Controls Odor Sensitivity in Larvae of Xenopus Laevis." *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* 30 (26): 8965–73. https://doi.org/10.1523/JNEUROSCI.4030-09.2010.

Brown, Alan S, Karin Borgmann-Winter, Chang-Gyu Hahn, Lorna Role, David Talmage, Raquel Gur, Jacky Chow, et al. 2014. "Increased Stability of Microtubules in Cultured Olfactory Neuroepithelial Cells from Individuals with Schizophrenia." *Progress in Neuro-Psychopharmacology & Biological Psychiatry* 48 (January): 252–58. https://doi.org/10.1016/j.pnpbp.2013.10.015.

Brunette, Mary F, Ree Dawson, Christopher D O'Keefe, Meera Narasimhan, Douglas L Noordsy, Joanne

Wojcik, and Alan I Green. 2011. “A Randomized Trial of Clozapine vs. Other Antipsychotics for Cannabis Use Disorder in Patients with Schizophrenia.” *Journal of Dual Diagnosis* 7 (1–2): 50–63. https://doi.org/10.1080/15504263.2011.570118.

Buchanan, Robert W., Julie Kreyenbuhl, Deanna L. Kelly, Jason M. Noel, Douglas L. Boggs, Bernard A. Fischer, Seth Himelhoch, et al. 2010. “The 2009 Schizophrenia PORT Psychopharmacological Treatment Recommendations and Summary Statements.” *Schizophrenia Bulletin* 36 (1): 71–93. https://doi.org/10.1093/schbul/sbp116.

Buchanan, Robert W. 2007. “Persistent Negative Symptoms in Schizophrenia: An Overview.” *Schizophrenia Bulletin* 33 (4): 1013–22. https://doi.org/10.1093/schbul/sbl057.

Calabrò, Rocco S, Alberto Cacciola, Daniele Bruschetta, Demetrio Milardi, Fabrizio Quattrini, Francesca Sciarrone, Gianluca la Rosa, Placido Bramanti, and Giuseppe Anastasi. 2019. “Neuroanatomy and Function of Human Sexual Behavior: A Neglected or Unknown Issue?” *Brain and Behavior* 9 (12): e01389. https://doi.org/10.1002/brb3.1389.

Castro-de-Araujo, Luis F S, Mathew Allin, Marco M Picchioni, Colm Mcdonald, Christos Pantelis, and Richard A A Kanaan. 2018. “Schizophrenia Moderates the Relationship between White Matter Integrity and Cognition.” *Schizophrenia Research* 199 (September): 250–56. https://doi.org/10.1016/j.schres.2018.03.033.

Ceccarini, Jenny, Marc De Hert, Ruud Van Winkel, Joseph Peuskens, Guy Bormans, Laura Kranaster, Frank Enning, Dagmar Koethe, F Markus Leweke, and Koen Van Laere. 2013. “Increased Ventral Striatal CB1 Receptor Binding Is Related to Negative Symptoms in Drug-Free Patients with Schizophrenia.” *NeuroImage* 79 (October): 304–12. https://doi.org/10.1016/j.neuroimage.2013.04.052.

Ceccarini, Jenny, Rebecca Kuepper, Dieter Kemels, Jim van Os, Cécile Henquet, and Koen Van Laere. 2015. “[18F]MK-9470 PET Measurement of Cannabinoid CB1 Receptor Availability in Chronic Cannabis Users.” *Addiction Biology* 20 (2): 357–67. https://doi.org/10.1111/adb.12116.

Cerdá, Magdalena, Melanie Wall, Tianshu Feng, Katherine M Keyes, Aaron Sarvet, John Schulenberg, Patrick M O’Malley, Rosalie Liccardo Pacula, Sandro Galea, and Deborah S Hasin. 2017. “Association of State Recreational Marijuana Laws With Adolescent Marijuana Use.” *JAMA Pediatrics* 171 (2): 142–49. https://doi.org/10.1001/jamapediatrics.2016.3624.

Chan, Raymond C K, and Irving I Gottesman. 2008. “Neurological Soft Signs as Candidate Endophenotypes for Schizophrenia: A Shooting Star or a Northern Star?” *Neuroscience and Biobehavioral Reviews* 32 (5): 957–71. https://doi.org/10.1016/j.neubiorev.2008.01.005.

Chan, Raymond C K, Ting Xu, R Walter Heinrichs, Yue Yu, and Ya Wang. 2010. “Neurological Soft Signs in Schizophrenia: A Meta-Analysis.” *Schizophrenia Bulletin* 36 (6): 1089–1104. https://doi.org/10.1093/schbul/sbp011.

Choi, Kee-Hong, Til Wykes, and Matthew M Kurtz. 2013. “Adjunctive Pharmacotherapy for Cognitive Deficits in Schizophrenia: Meta-Analytical Investigation of Efficacy.” *The British Journal of Psychiatry : The Journal of Mental Science* 203 (3): 172–78. https://doi.org/10.1192/bjp.bp.111.107359.

Chong, Huey Yi, Siew Li Teoh, David Bin-Chia Wu, Surachai Kotirum, Chiun-Fang Chiou, and Nathorn Chaiyakunapruk. 2016. "Global Economic Burden of Schizophrenia: A Systematic Review." *Neuropsychiatric Disease and Treatment* 12: 357–73. https://doi.org/10.2147/NDT.S96649.

Ciurleo, Rosella, Lilla Bonanno, Simona De Salvo, Laura Romeo, Carmela Rifici, Edoardo Sessa, Giangaetano D'Aleo, et al. 2018. "Olfactory Dysfunction as a Prognostic Marker for Disability Progression in Multiple Sclerosis: An Olfactory Event Related Potential Study." *PloS One* 13 (4): e0196006. https://doi.org/10.1371/journal.pone.0196006.

Correll, C U. 2010. "From Receptor Pharmacology to Improved Outcomes: Individualising the Selection, Dosing, and Switching of Antipsychotics." *European Psychiatry : The Journal of the Association of European Psychiatrists* 25 Suppl 2 (June): S12-21. https://doi.org/10.1016/S0924-9338(10)71701-6.

Coyle, Joseph T, Darrick Balu, Michael Benneyworth, Alo Basu, and Alexander Roseman. 2010. "Beyond the Dopamine Receptor: Novel Therapeutic Targets for Treating Schizophrenia." *Dialogues in Clinical Neuroscience* 12 (3): 359–82.

Crean, Rebecca D, Susan F Tapert, Arpi Minassian, Kai Macdonald, Natania A Crane, and Barbara J Mason. 2011. "Effects of Chronic, Heavy Cannabis Use on Executive Functions." *Journal of Addiction Medicine* 5 (1): 9–15. https://doi.org/10.1097/ADM.0b013e31820cdd57.

Crespo-Facorro, Benedicto, Jose Maria Pelayo-Teran, and Jacqueline Mayoral-van Son. 2016. "Current Data on and Clinical Insights into the Treatment of First Episode Nonaffective Psychosis: A Comprehensive Review." *Neurology and Therapy* 5 (2): 105–30. https://doi.org/10.1007/s40120-016-0050-8.

Crespo-Facorro, Benedicto, Pedro Such, Anna-Greta Nylander, Jessica Madera, Henrike K Resemann, Emma Worthington, Mary-Ellen O'Connor, Emma Drane, Sara Steeves, and Richard Newton. 2020. "The Burden of Disease in Early Schizophrenia - a Systematic Literature Review." *Current Medical Research and Opinion*, October, 1. https://doi.org/10.1080/03007995.2020.1841618.

Cryan, J F, A Harkin, M Naughton, J P Kelly, and B E Leonard. 2000. "Characterization of D-Fenfluramine-Induced Hypothermia: Evidence for Multiple Sites of Action." *European Journal of Pharmacology* 390 (3): 275–85. https://doi.org/10.1016/s0014-2999(00)00012-1.

D'Souza, Deepak Cyril, Jose A. Cortes-Briones, Mohini Ranganathan, Halle Thurnauer, Gina Creatura, Toral Surti, Beata Planeta, et al. 2016. "Rapid Changes in Cannabinoid 1 Receptor Availability in Cannabis-Dependent Male Subjects After Abstinence From Cannabis." *Biological Psychiatry: Cognitive Neuroscience and Neuroimaging* 1 (1): 60–67. https://doi.org/10.1016/j.bpsc.2015.09.008.

D'Souza, Deepak Cyril, Suhas Ganesh, Jose Cortes-Briones, Michael H Campbell, and Maisha K Emmanuel. 2019. "Characterizing Psychosis-Relevant Phenomena and Cognitive Function in a Unique Population with Isolated, Chronic and Very Heavy Cannabis Exposure." *Psychological Medicine*, October, 1–8. https://doi.org/10.1017/S0033291719002721.

D'Souza, Deepak Cyril, Edward Perry, Lisa MacDougall, Yola Ammerman, Thomas Cooper, Yu Te Wu, Gabriel Braley, Ralitza Gueorguieva, and John Harrison Krystal. 2004. "The Psychotomimetic Effects

of Intravenous Delta-9-Tetrahydrocannabinol in Healthy Individuals: Implications for Psychosis." *Neuropsychopharmacology* 29 (8): 1558–72. https://doi.org/10.1038/sj.npp.1300496.

Dalton, Victoria S, Leonora E Long, Cyndi Shannon Weickert, and Katerina Zavitsanou. 2011. "Paranoid Schizophrenia Is Characterized by Increased CB1 Receptor Binding in the Dorsolateral Prefrontal Cortex." *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology* 36 (8): 1620–30. https://doi.org/10.1038/npp.2011.43.

Darmani, N A. 2001. "Cannabinoids of Diverse Structure Inhibit Two DOI-Induced 5-HT(2A) Receptor-Mediated Behaviors in Mice." *Pharmacology, Biochemistry, and Behavior* 68 (2): 311–17.

Davidson, Michael, Silvana Galderisi, Mark Weiser, Nomi Werbeloff, Wolfgang W Fleischhacker, Richard S Keefe, Han Boter, et al. 2009. "Cognitive Effects of Antipsychotic Drugs in First-Episode Schizophrenia and Schizophreniform Disorder: A Randomized, Open-Label Clinical Trial (EUFEST)." *The American Journal of Psychiatry* 166 (6): 675–82. https://doi.org/10.1176/appi.ajp.2008.08060806.

Dazzan, Paola, and Robin M Murray. 2002. "Neurological Soft Signs in First-Episode Psychosis: A Systematic Review." *The British Journal of Psychiatry. Supplement* 43 (September): s50-7. https://doi.org/10.1192/bjp.181.43.s50.

Desamericq, G, F Schurhoff, A Meary, A Szoke, I Macquin-Mavier, A C Bachoud-Levi, and P Maison. 2014. "Long-Term Neurocognitive Effects of Antipsychotics in Schizophrenia: A Network Meta-Analysis." *European Journal of Clinical Pharmacology* 70 (2): 127–34. https://doi.org/10.1007/s00228-013-1600-y.

Devane, W A, L Hanus, A Breuer, R G Pertwee, L A Stevenson, G Griffin, D Gibson, A Mandelbaum, A Etinger, and R Mechoulam. 1992. "Isolation and Structure of a Brain Constituent That Binds to the Cannabinoid Receptor." *Science (New York, N.Y.)* 258 (5090): 1946–49. https://doi.org/10.1126/science.1470919.

Devlin, Mark G, and Arthur Christopoulos. 2002. "Modulation of Cannabinoid Agonist Binding by 5-HT in the Rat Cerebellum." *Journal of Neurochemistry* 80 (6): 1095–1102. https://doi.org/10.1046/j.0022-3042.2002.00797.x.

Dienel, Samuel J, and David A Lewis. 2019. "Alterations in Cortical Interneurons and Cognitive Function in Schizophrenia." *Neurobiology of Disease* 131 (November): 104208. https://doi.org/10.1016/j.nbd.2018.06.020.

Dursun, S M, A Szemis, H Andrews, P Whitaker, and M A Reveley. 2000. "Effects of Clozapine and Typical Antipsychotic Drugs on Plasma 5-HT Turnover and Impulsivity in Patients with Schizophrenia: A Cross-Sectional Study." *Journal of Psychiatry & Neuroscience : JPN* 25 (4): 347–52.

Eggan, Stephen M, Takanori Hashimoto, and David A Lewis. 2008. "Reduced Cortical Cannabinoid 1 Receptor Messenger RNA and Protein Expression in Schizophrenia." *Archives of General Psychiatry* 65 (7): 772–84. https://doi.org/10.1001/archpsyc.65.7.772.

Eggan, Stephen M, Samuel R Stoyak, Christopher D Verrico, and David A Lewis. 2010. "Cannabinoid CB1 Receptor Immunoreactivity in the Prefrontal Cortex: Comparison of Schizophrenia and Major

Depressive Disorder." *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology* 35 (10): 2060–71. https://doi.org/10.1038/npp.2010.75.

Eggers, Arnold E. 2012. "Extending David Horrobin's Membrane Phospholipid Theory of Schizophrenia: Overactivity of Cytosolic Phospholipase A(2) in the Brain Is Caused by Overdrive of Coupled Serotonergic 5HT(2A/2C) Receptors in Response to Stress." *Medical Hypotheses* 79 (6): 740–43. https://doi.org/10.1016/j.mehy.2012.08.016.

Eggers, Arnold E. 2013. "A Serotonin Hypothesis of Schizophrenia." *Medical Hypotheses* 80 (6): 791–94. https://doi.org/10.1016/j.mehy.2013.03.013.

English, J. A., Y. Fan, M. Föcking, L. M. Lopez, M. Hryniewiecka, K. Wynne, P. Dicker, et al. 2015. "Reduced Protein Synthesis in Schizophrenia Patient-Derived Olfactory Cells." *Translational Psychiatry* 5 (July): e663. https://doi.org/10.1038/tp.2015.119.

European Monitoring Centre for Drugs and Drug Addiction. 2018. "European Drug Report." 2018. https://www.emcdda.europa.eu/edr2018.

Evensen, Julie, Jan Ivar Røssberg, Helene Barder, Ulrik Haahr, Wenche ten Velden Hegelstad, Inge Joa, Jan Olav Johannessen, et al. 2012. "Apathy in First Episode Psychosis Patients: A Ten Year Longitudinal Follow-up Study." *Schizophrenia Research* 136 (1–3): 19–24. https://doi.org/10.1016/j.schres.2011.12.019.

Fan, Yongjun, Greger Abrahamsen, John J McGrath, and Alan Mackay-Sim. 2012. "Altered Cell Cycle Dynamics in Schizophrenia." *Biological Psychiatry* 71 (2): 129–35. https://doi.org/10.1016/j.biopsych.2011.10.004.

Farrer, Louise M, Jennie Walker, Christopher Harrison, and Michelle Banfield. 2018. "Primary Care Access for Mental Illness in Australia: Patterns of Access to General Practice from 2006 to 2016." *PloS One* 13 (6): e0198400. https://doi.org/10.1371/journal.pone.0198400.

Fatouros-Bergman, Helena, Simon Cervenka, Lena Flyckt, Gunnar Edman, and Lars Farde. 2014. "Meta-Analysis of Cognitive Performance in Drug-Naïve Patients with Schizophrenia." *Schizophrenia Research* 158 (1–3): 156–62. https://doi.org/10.1016/j.schres.2014.06.034.

Fernandez-Ruiz, Javier. 2009. "The Endocannabinoid System as a Target for the Treatment of Motor Dysfunction." *British Journal of Pharmacology* 156 (7): 1029–40. https://doi.org/10.1111/j.1476-5381.2008.00088.x.

Ferré, Sergi, Vicent Casadó, Lakshmi A Devi, Marta Filizola, Ralf Jockers, Martin J Lohse, Graeme Milligan, Jean Philippe Pin, and Xavier Guitart. 2014. "G Protein-Coupled Receptor Oligomerization Revisited: Functional and Pharmacological Perspectives." *Pharmacological Reviews*. https://doi.org/10.1124/pr.113.008052.

Fervaha, G, G Foussias, O Agid, and G Remington. 2014. "Impact of Primary Negative Symptoms on Functional Outcomes in Schizophrenia." *European Psychiatry : The Journal of the Association of European Psychiatrists* 29 (7): 449–55. https://doi.org/10.1016/j.eurpsy.2014.01.007.

Filbey, Francesca, and Uma Yezhuvath. 2013. "Functional Connectivity in Inhibitory Control Networks and Severity of Cannabis Use Disorder." *The American Journal of Drug and Alcohol Abuse* 39 (6): 382–

91. https://doi.org/10.3109/00952990.2013.841710.

Flores, Gonzalo, Julio Cesar Morales-Medina, and Alfonso Diaz. 2016. "Neuronal and Brain Morphological Changes in Animal Models of Schizophrenia." *Behavioural Brain Research* 301 (March): 190–203. https://doi.org/10.1016/j.bbr.2015.12.034.

Ford, Talitha C, Amie C Hayley, Luke A Downey, and Andrew C Parrott. 2017. "Cannabis: An Overview of Its Adverse Acute and Chronic Effects and Its Implications." *Current Drug Abuse Reviews* 10 (1): 6–18. https://doi.org/10.2174/1874473710666170712113042.

Fortenbaugh, Francesca C, David Rothlein, Regina McGlinchey, Joseph DeGutis, and Michael Esterman. 2018. "Tracking Behavioral and Neural Fluctuations during Sustained Attention: A Robust Replication and Extension." *NeuroImage* 171 (May): 148–64. https://doi.org/10.1016/j.neuroimage.2018.01.002.

Forti, Marta Di, Diego Quattrone, Tom P Freeman, Giada Tripoli, Charlotte Gayer-Anderson, Harriet Quigley, Victoria Rodriguez, et al. 2019. "The Contribution of Cannabis Use to Variation in the Incidence of Psychotic Disorder across Europe (EU-GEI): A Multicentre Case-Control Study." *The Lancet. Psychiatry* 6 (5): 427–36. https://doi.org/10.1016/S2215-0366(19)30048-3.

Forti, Marta Di, Hannah Sallis, Fabio Allegri, Antonella Trotta, Laura Ferraro, Simona A Stilo, Arianna Marconi, et al. 2014. "Daily Use, Especially of High-Potency Cannabis, Drives the Earlier Onset of Psychosis in Cannabis Users." *Schizophrenia Bulletin* 40 (6): 1509–17. https://doi.org/10.1093/schbul/sbt181.

Franklin, Jade M, and Gonzalo A Carrasco. 2013. "Cannabinoid Receptor Agonists Upregulate and Enhance Serotonin 2A (5-HT(2A)) Receptor Activity via ERK1/2 Signaling." *Synapse (New York, N.Y.)* 67 (3): 145–59. https://doi.org/10.1002/syn.21626.

Fusar-Poli, Paolo, Patrick D McGorry, and John M. Kane. 2017. "Improving Outcomes of First-Episode Psychosis: An Overview.". *World Psychiatry* 16 (3): 251–65. https://doi.org/http://dx.doi.org/10.1002/wps.20446.

Fuxe, K, L F Agnati, F Benfenati, M Celani, I Zini, M Zoli, and V Mutt. 1983. "Evidence for the Existence of Receptor--Receptor Interactions in the Central Nervous System. Studies on the Regulation of Monoamine Receptors by Neuropeptides." *Journal of Neural Transmission. Supplementum* 18: 165–79.

Gage, Suzanne H, Matthew Hickman, and Stanley Zammit. 2016. "Association Between Cannabis and Psychosis: Epidemiologic Evidence." *Biological Psychiatry* 79 (7): 549–56. https://doi.org/10.1016/j.biopsych.2015.08.001.

Gao, Yonglin, Welby Winstead, Zhenmin Lei, Chengliang Lu, Fred J. Roisen, and Rif S. El-Mallakh. 2017. "Olfactory Neuroepithelial Neural Progenitor Cells from Subjects with Bipolar I Disorder." *Journal of Central Nervous System Disease* 9: 117957351769452. https://doi.org/10.1177/1179573517694529.

García-Bea, Aintzane, Patricia Miranda-Azpiazu, Carolina Muguruza, Sara Marmolejo-Martinez-Artesero, Rebeca Diez-Alarcia, Ane M Gabilondo, Luis F Callado, Benito Morentin, Javier González-Maeso,

and J Javier Meana. 2019. “Serotonin 5-HT(2A) Receptor Expression and Functionality in Postmortem Frontal Cortex of Subjects with Schizophrenia: Selective Biased Agonism via G(Ai1)-Proteins.” *European Neuropsychopharmacology : The Journal of the European College of Neuropsychopharmacology* 29 (12): 1453–63. https://doi.org/10.1016/j.euroneuro.2019.10.013.

Genovese, Giulio, Menachem Fromer, Eli A Stahl, Douglas M Ruderfer, Kimberly Chambert, Mikael Landén, Jennifer L Moran, et al. 2016. “Increased Burden of Ultra-Rare Protein-Altering Variants among 4,877 Individuals with Schizophrenia.” *Nature Neuroscience* 19 (11): 1433–41. https://doi.org/10.1038/nn.4402.

Golimbet, V E, O M Lavrushina, V G Kaleda, L I Abramova, and T V Lezheiko. 2007. “Supportive Evidence for the Association between the T102C 5-HTR2A Gene Polymorphism and Schizophrenia: A Large-Scale Case-Control and Family-Based Study.” *European Psychiatry : The Journal of the Association of European Psychiatrists* 22 (3): 167–70. https://doi.org/10.1016/j.eurpsy.2006.06.002.

Goodman, Jarid, and Mark G Packard. 2015. “The Influence of Cannabinoids on Learning and Memory Processes of the Dorsal Striatum.” *Neurobiology of Learning and Memory* 125 (November): 1–14. https://doi.org/10.1016/j.nlm.2015.06.008.

Gourion, D, C Goldberger, J P Olie, H Lôo, and M O Krebs. 2004. “Neurological and Morphological Anomalies and the Genetic Liability to Schizophrenia: A Composite Phenotype.” *Schizophrenia Research* 67 (1): 23–31. https://doi.org/10.1016/s0920-9964(03)00099-9.

Green, Alan I, Ellen S Burgess, Ree Dawson, Suzannah V Zimmet, and Rael D Strous. 2003. “Alcohol and Cannabis Use in Schizophrenia: Effects of Clozapine vs. Risperidone.” *Schizophrenia Research* 60 (1): 81–85. https://doi.org/10.1016/s0920-9964(02)00231-1.

Green, Alan I, Douglas L Noordsy, Mary F Brunette, and Christopher O’Keefe. 2008. “Substance Abuse and Schizophrenia: Pharmacotherapeutic Intervention.” *Journal of Substance Abuse Treatment* 34 (1): 61–71. https://doi.org/10.1016/j.jsat.2007.01.008.

Green, Michael F, Robert S Kern, and Robert K Heaton. 2004. “Longitudinal Studies of Cognition and Functional Outcome in Schizophrenia: Implications for MATRICS.” *Schizophrenia Research* 72 (1): 41–51. https://doi.org/10.1016/j.schres.2004.09.009.

Guinart, Daniel, and Christoph U Correll. 2020. “Antipsychotic Polypharmacy in Schizophrenia: Why Not?” *The Journal of Clinical Psychiatry* 81 (3). https://doi.org/10.4088/JCP.19ac13118.

Gunasekaran, Vanishree, V Mathan Kumar Venkatesh, and T V Asokan. 2016. “A Study of Soft Neurological Signs and Its Correlates in Drug-Naive Patients with First Episode Psychosis.” *Indian Journal of Psychological Medicine* 38 (5): 408–13. https://doi.org/10.4103/0253-7176.191393.

Haj-Dahmane, Samir, and Roh-Yu Shen. 2011. “Modulation of the Serotonin System by Endocannabinoid Signaling.” *Neuropharmacology* 61 (3): 414–20. https://doi.org/10.1016/j.neuropharm.2011.02.016.

Hall, Wayne, and Louisa Degenhardt. 2009. “Adverse Health Effects of Non-Medical Cannabis Use.” *Lancet (London, England)* 374 (9698): 1383–91. https://doi.org/10.1016/S0140-6736(09)61037-0.

Hannon, Jason, and Daniel Hoyer. 2008. “Molecular Biology of 5-HT Receptors.” *Behavioural Brain*

Research 195 (1): 198–213. https://doi.org/10.1016/j.bbr.2008.03.020.

Hardy, A, B Palouzier-Paulignan, A Duchamp, J-P Royet, and P Duchamp-Viret. 2005. "5-Hydroxytryptamine Action in the Rat Olfactory Bulb: In Vitro Electrophysiological Patch-Clamp Recordings of Juxtaglomerular and Mitral Cells." *Neuroscience* 131 (3): 717–31. https://doi.org/10.1016/j.neuroscience.2004.10.034.

Hari, Churunal, Ben Grimshaw, and Tim Jacob. 2018. "Effect of Lidocaine on Olfactory Perception in Humans." *International Journal of Applied & Basic Medical Research* 8 (3): 164–68. https://doi.org/10.4103/ijabmr.IJABMR_2_18.

Häring, Martin, Vanessa Enk, Alejandro Aparisi Rey, Sebastian Loch, Inigo Ruiz de Azua, Tillmann Weber, Dusan Bartsch, Krisztina Monory, and Beat Lutz. 2015. "Cannabinoid Type-1 Receptor Signaling in Central Serotonergic Neurons Regulates Anxiety-like Behavior and Sociability." *Frontiers in Behavioral Neuroscience* 9: 235. https://doi.org/10.3389/fnbeh.2015.00235.

Harvey, Philip D, and R S Keefe. 2001. "Studies of Cognitive Change in Patients with Schizophrenia Following Novel Antipsychotic Treatment." *The American Journal of Psychiatry* 158 (2): 176–84. https://doi.org/10.1176/appi.ajp.158.2.176.

Harvey, Philip D. 2009. "Pharmacological Cognitive Enhancement in Schizophrenia." *Neuropsychology Review* 19 (3): 324–35. https://doi.org/10.1007/s11065-009-9103-4.

Hasbi, Ahmed, Bertha K Madras, Jack Bergman, Stephen Kohut, Zhicheng Lin, Sarah L Withey, and Susan R George. 2020. "Delta-Tetrahydrocannabinol Increases Dopamine D1-D2 Receptor Heteromer and Elicits Phenotypic Reprogramming in Adult Primate Striatal Neurons." *IScience* 23 (1): 100794. https://doi.org/10.1016/j.isci.2019.100794.

Hayes, Joseph F, Louise Marston, Kate Walters, Michael B King, and David P J Osborn. 2017. "Mortality Gap for People with Bipolar Disorder and Schizophrenia: UK-Based Cohort Study 2000-2014." *The British Journal of Psychiatry : The Journal of Mental Science* 211 (3): 175–81. https://doi.org/10.1192/bjp.bp.117.202606.

Hert, Marc DE, Christoph U Correll, Julio Bobes, Marcelo Cetkovich-Bakmas, Dan Cohen, Itsuo Asai, Johan Detraux, et al. 2011. "Physical Illness in Patients with Severe Mental Disorders. I. Prevalence, Impact of Medications and Disparities in Health Care." *World Psychiatry : Official Journal of the World Psychiatric Association (WPA)* 10 (1): 52–77. https://doi.org/10.1002/j.2051-5545.2011.tb00014.x.

Hill, Jeremy D, Viviana Zuluaga-Ramirez, Sachin Gajghate, Malika Winfield, Uma Sriram, Slava Rom, and Yuri Persidsky. 2019. "Activation of GPR55 Induces Neuroprotection of Hippocampal Neurogenesis and Immune Responses of Neural Stem Cells Following Chronic, Systemic Inflammation." *Brain, Behavior, and Immunity* 76 (February): 165–81. https://doi.org/10.1016/j.bbi.2018.11.017.

Hirvonen, J, R S Goodwin, C-T Li, G E Terry, S S Zoghbi, C Morse, V W Pike, N D Volkow, M A Huestis, and R B Innis. 2012. "Reversible and Regionally Selective Downregulation of Brain Cannabinoid CB1 Receptors in Chronic Daily Cannabis Smokers." *Molecular Psychiatry* 17 (6): 642–49. https://doi.org/10.1038/mp.2011.82.

Hjorthøj, Carsten, Anne Emilie Stürup, John J McGrath, and Merete Nordentoft. 2017. "Years of Potential Life Lost and Life Expectancy in Schizophrenia: A Systematic Review and Meta-Analysis." *The Lancet. Psychiatry* 4 (4): 295–301. https://doi.org/10.1016/S2215-0366(17)30078-0.

Holm, Minna, Heidi Taipale, Antti Tanskanen, Jari Tiihonen, and Ellenor Mitterdorfer-Rutz. 2020. "Employment among People with Schizophrenia or Bipolar Disorder: A Population-Based Study Using Nationwide Registers." *Acta Psychiatrica Scandinavica*, November. https://doi.org/10.1111/acps.13254.

Hor, Kahyee, and Mark Taylor. 2010. "Suicide and Schizophrenia: A Systematic Review of Rates and Risk Factors." *Journal of Psychopharmacology (Oxford, England)* 24 (4 Suppl): 81–90. https://doi.org/10.1177/1359786810385490.

Horiuchi, Y., M. A. Kondo, K. Okada, Y. Takayanagi, T. Tanaka, T. Ho, M. Varvaris, et al. 2016. "Molecular Signatures Associated with Cognitive Deficits in Schizophrenia: A Study of Biopsied Olfactory Neural Epithelium." *Translational Psychiatry* 6 (10): e915. https://doi.org/10.1038/tp.2016.154.

Horiuchi, Yasue, Shin-Ichi Kano, Koko Ishizuka, Nicola G Cascella, Seiji Ishii, C Conover Jr Talbot, Andrew E Jaffe, et al. 2013. "Olfactory Cells via Nasal Biopsy Reflect the Developing Brain in Gene Expression Profiles: Utility and Limitation of the Surrogate Tissues in Research for Brain Disorders." *Neuroscience Research* 77 (4): 247–50. https://doi.org/10.1016/j.neures.2013.09.010.

Hornung, Jean-Pierre. 2003. "The Human Raphe Nuclei and the Serotonergic System." *Journal of Chemical Neuroanatomy* 26 (4): 331–43. https://doi.org/10.1016/j.jchemneu.2003.10.002.

Howes, Oliver D, Robert McCutcheon, Michael J Owen, and Robin M Murray. 2017. "The Role of Genes, Stress, and Dopamine in the Development of Schizophrenia." *Biological Psychiatry* 81 (1): 9–20. https://doi.org/10.1016/j.biopsych.2016.07.014.

Howlett, Allyn C, Lawrence C Blume, and George D Dalton. 2010. "CB(1) Cannabinoid Receptors and Their Associated Proteins." *Current Medicinal Chemistry* 17 (14): 1382–93. https://doi.org/10.2174/092986710790980023.

Hoyer, Daniel, Jason P Hannon, and Graeme R Martin. 2002. "Molecular, Pharmacological and Functional Diversity of 5-HT Receptors." *Pharmacology, Biochemistry, and Behavior* 71 (4): 533–54. https://doi.org/10.1016/s0091-3057(01)00746-8.

Huestis, Marilyn A, Jack E Henningfield, and Edward J Cone. 1992. "Blood Cannabinoids. i. Absorption of Thc and Formation of 11-Oh-Thc and Thccooh during and after Smoking Marijuana." *Journal of Analytical Toxicology* 16 (5): 276–82. https://doi.org/10.1093/jat/16.5.276.

Huhn, Maximilian, Adriani Nikolakopoulou, Johannes Schneider-Thoma, Marc Krause, Myrto Samara, Natalie Peter, Thomas Arndt, et al. 2019. "Comparative Efficacy and Tolerability of 32 Oral Antipsychotics for the Acute Treatment of Adults with Multi-Episode Schizophrenia: A Systematic Review and Network Meta-Analysis." *Lancet (London, England)*, July. https://doi.org/10.1016/S0140-6736(19)31135-3.

Hunt, Glenn E, Matthew M Large, Michelle Cleary, Harry Man Xiong Lai, and John B Saunders. 2018. "Prevalence of Comorbid Substance Use in Schizophrenia Spectrum Disorders in Community and

Clinical Settings, 1990-2017: Systematic Review and Meta-Analysis." *Drug and Alcohol Dependence* 191 (October): 234–58. https://doi.org/10.1016/j.drugalcdep.2018.07.011.

Hurlemann, René, Andreas Matusch, Kai-Uwe Kuhn, Julia Berning, David Elmenhorst, Oliver Winz, Heike Kolsch, et al. 2008. "5-HT2A Receptor Density Is Decreased in the at-Risk Mental State." *Psychopharmacology* 195 (4): 579–90. https://doi.org/10.1007/s00213-007-0921-x.

Husa, Anja P, Jani Moilanen, Graham K Murray, Riikka Marttila, Marianne Haapea, Irina Rannikko, Jennifer H Barnett, et al. 2017. "Lifetime Antipsychotic Medication and Cognitive Performance in Schizophrenia at Age 43 Years in a General Population Birth Cohort." *Psychiatry Research* 247 (January): 130–38. https://doi.org/10.1016/j.psychres.2016.10.085.

Hutch, C R, C J Hillard, C Jia, and C C Hegg. 2015. "An Endocannabinoid System Is Present in the Mouse Olfactory Epithelium but Does Not Modulate Olfaction." *Neuroscience* 300 (August): 539–53. https://doi.org/10.1016/j.neuroscience.2015.05.056.

Ibarra-Lecue, Ines, Irene Mollinedo-Gajate, J Javier Meana, Luis F Callado, Rebeca Diez-Alarcia, and Leyre Uriguen. 2018. "Chronic Cannabis Promotes Pro-Hallucinogenic Signaling of 5-HT2A Receptors through Akt/MTOR Pathway." *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology* 43 (10): 2028–35. https://doi.org/10.1038/s41386-018-0076-y.

Ibarra-Lecue, Inés, Fuencisla Pilar-Cuéllar, Carolina Muguruza, Eva Florensa-Zanuy, Álvaro Díaz, Leyre Urigüen, Elena Castro, Angel Pazos, and Luis F Callado. 2018. "The Endocannabinoid System in Mental Disorders: Evidence from Human Brain Studies." *Biochemical Pharmacology* 157 (November): 97–107. https://doi.org/10.1016/j.bcp.2018.07.009.

Idotta, Carlo, Elena Tibaldi, Anna Maria Brunati, Mario Angelo Pagano, Massimiliano Cadamuro, Alessandro Miola, Alessandro Martini, et al. 2019. "Olfactory Neuroepithelium Alterations and Cognitive Correlates in Schizophrenia." *European Psychiatry* 61: 23–32. https://doi.org/10.1016/j.eurpsy.2019.06.004.

Igolkina, Anna A., Chris Armoskus, Jeremy R.B. Newman, Oleg V. Evgrafov, Lauren M. McIntyre, Sergey V. Nuzhdin, and Maria G. Samsonova. 2018. "Analysis of Gene Expression Variance in Schizophrenia Using Structural Equation Modeling." *Frontiers in Molecular Neuroscience* 11 (June): 1–12. https://doi.org/10.3389/fnmol.2018.00192.

Insel, Thomas R. 2010. "Rethinking Schizophrenia." *Nature* 468 (7321): 187–93. https://doi.org/10.1038/nature09552.

Jenko, Kimberly J, Jussi Hirvonen, Ioline D Henter, Kacey B Anderson, Sami S Zoghbi, Thomas M Hyde, Amy Deep-Soboslay, Robert B Innis, and Joel E Kleinman. 2012. "Binding of a Tritiated Inverse Agonist to Cannabinoid CB1 Receptors Is Increased in Patients with Schizophrenia." *Schizophrenia Research* 141 (2–3): 185–88. https://doi.org/10.1016/j.schres.2012.07.021.

Jiménez-Vaca, Ana L., Gloria Benitez-King, Víctor Ruiz, Gerardo B. Ramírez-Rodríguez, Beatriz Hernández-de la Cruz, Fabio A. Salamanca-Gómez, Humberto González-Márquez, et al. 2018. "Exfoliated Human Olfactory Neuroepithelium: A Source of Neural Progenitor Cells." *Molecular Neurobiology*

55 (3): 2516–23. https://doi.org/10.1007/s12035-017-0500-z.

Jónsdóttir, H, S Opjordsmoen, A B Birkenaes, C Simonsen, J A Engh, P A Ringen, A Vaskinn, S Friis, K Sundet, and O A Andreassen. 2013. "Predictors of Medication Adherence in Patients with Schizophrenia and Bipolar Disorder." *Acta Psychiatrica Scandinavica* 127 (1): 23–33. https://doi.org/10.1111/j.1600-0447.2012.01911.x.

Jung, Hahn Jin, In-Soo Shin, and Ji-Eun Lee. 2019. "Olfactory Function in Mild Cognitive Impairment and Alzheimer's Disease: A Meta-Analysis." *The Laryngoscope* 129 (2): 362–69. https://doi.org/10.1002/lary.27399.

Kane, John M, Ofer Agid, Marjorie L Baldwin, Oliver Howes, Jean-Pierre Lindenmayer, Stephen Marder, Mark Olfson, Steven G Potkin, and Christoph U Correll. 2019. "Clinical Guidance on the Identification and Management of Treatment-Resistant Schizophrenia." *The Journal of Clinical Psychiatry* 80 (2). https://doi.org/10.4088/JCP.18com12123.

Kane, John M, and Todd Lencz. 2008. "Cognitive Deficits in Schizophrenia: Short-Term and Long-Term." *World Psychiatry : Official Journal of the World Psychiatric Association (WPA)* 7 (1): 29–30.

Katsuki, Fumi, and Christos Constantinidis. 2014. "Bottom-up and Top-down Attention: Different Processes and Overlapping Neural Systems." *The Neuroscientist : A Review Journal Bringing Neurobiology, Neurology and Psychiatry* 20 (5): 509–21. https://doi.org/10.1177/1073858413514136.

Katzman, Martin A, Melissa Furtado, and Leena Anand. 2016. "Targeting the Endocannabinoid System in Psychiatric Illness." *Journal of Clinical Psychopharmacology* 36 (6): 691–703. https://doi.org/10.1097/JCP.0000000000000581.

Keating, Dolores, Stephen McWilliams, Ian Schneider, Caroline Hynes, Grainne Cousins, Judith Strawbridge, and Mary Clarke. 2017. "Pharmacological Guidelines for Schizophrenia: A Systematic Review and Comparison of Recommendations for the First Episode." *BMJ Open* 7 (1): e013881. https://doi.org/10.1136/bmjopen-2016-013881.

Keefe, Richard S E. 2014. "The Longitudinal Course of Cognitive Impairment in Schizophrenia: An Examination of Data from Premorbid through Posttreatment Phases of Illness." *The Journal of Clinical Psychiatry* 75 Suppl 2: 8–13. https://doi.org/10.4088/JCP.13065su1.02.

Keefe, Richard S E, Robert M Bilder, Sonia M Davis, Philip D Harvey, Barton W Palmer, James M Gold, Herbert Y Meltzer, et al. 2007. "Neurocognitive Effects of Antipsychotic Medications in Patients with Chronic Schizophrenia in the CATIE Trial." *Archives of General Psychiatry* 64 (6): 633–47. https://doi.org/10.1001/archpsyc.64.6.633.

Keefe, Richard S E, and Wayne S Fenton. 2007. "How Should DSM-V Criteria for Schizophrenia Include Cognitive Impairment?" *Schizophrenia Bulletin* 33 (4): 912–20. https://doi.org/10.1093/schbul/sbm046.

Keepers, George A, Laura J Fochtmann, Joan M Anzia, Sheldon Benjamin, Jeffrey M Lyness, Ramin Mojtabai, Mark Servis, et al. 2020. "The American Psychiatric Association Practice Guideline for the Treatment of Patients With Schizophrenia." *The American Journal of Psychiatry* 177 (9): 868–72.

https://doi.org/10.1176/appi.ajp.2020.177901.

Kirkpatrick, Brian, Wayne S Fenton, William T Jr Carpenter, and Stephen R Marder. 2006. "The NIMH-MATRICS Consensus Statement on Negative Symptoms." *Schizophrenia Bulletin* 32 (2): 214–19. https://doi.org/10.1093/schbul/sbj053.

Kirschner, Matthias, André Aleman, and Stefan Kaiser. 2017. "Secondary Negative Symptoms - A Review of Mechanisms, Assessment and Treatment." *Schizophrenia Research* 186 (August): 29–38. https://doi.org/10.1016/j.schres.2016.05.003.

Koblan, Kenneth S, Justine Kent, Seth C Hopkins, John H Krystal, Hailong Cheng, Robert Goldman, and Antony Loebel. 2020. "A Non-D2-Receptor-Binding Drug for the Treatment of Schizophrenia." *The New England Journal of Medicine* 382 (16): 1497–1506. https://doi.org/10.1056/NEJMoa1911772.

Koethe, D, I C Llenos, J R Dulay, C Hoyer, E F Torrey, F M Leweke, and S Weis. 2007. "Expression of CB1 Cannabinoid Receptor in the Anterior Cingulate Cortex in Schizophrenia, Bipolar Disorder, and Major Depression." *Journal of Neural Transmission (Vienna, Austria : 1996)* 114 (8): 1055–63. https://doi.org/10.1007/s00702-007-0660-5.

Kremen, William S, Larry J Seidman, Stephen V Faraone, Rosemary Toomey, and Ming T Tsuang. 2004. "Heterogeneity of Schizophrenia: A Study of Individual Neuropsychological Profiles." *Schizophrenia Research* 71 (2–3): 307–21. https://doi.org/10.1016/j.schres.2004.02.022.

Kugathasan, Pirathiv, Henriette Thisted Horsdal, Jørgen Aagaard, Svend Eggert Jensen, Thomas Munk Laursen, and René Ernst Nielsen. 2018. "Association of Secondary Preventive Cardiovascular Treatment After Myocardial Infarction With Mortality Among Patients With Schizophrenia." *JAMA Psychiatry* 75 (12): 1234–40. https://doi.org/10.1001/jamapsychiatry.2018.2742.

Kugathasan, Pirathiv, Honghan Wu, Fiona Gaughran, René Ernst Nielsen, Megan Pritchard, Richard Dobson, Robert Stewart, and Brendon Stubbs. 2020. "Association of Physical Health Multimorbidity with Mortality in People with Schizophrenia Spectrum Disorders: Using a Novel Semantic Search System That Captures Physical Diseases in Electronic Patient Records." *Schizophrenia Research* 216 (February): 408–15. https://doi.org/10.1016/j.schres.2019.10.061.

Kusumi, Ichiro, Shuken Boku, and Yoshito Takahashi. 2015. "Psychopharmacology of Atypical Antipsychotic Drugs: From the Receptor Binding Profile to Neuroprotection and Neurogenesis." *Psychiatry and Clinical Neurosciences* 69 (5): 243–58. https://doi.org/10.1111/pcn.12242.

Lafaye, Genevieve, Laurent Karila, Lisa Blecha, and Amine Benyamina. 2017. "Cannabis, Cannabinoids, and Health." *Dialogues in Clinical Neuroscience* 19 (3): 309–16. https://doi.org/10.31887/DCNS.2017.19.3/glafaye.

Langner, Robert, and Simon B Eickhoff. 2013. "Sustaining Attention to Simple Tasks: A Meta-Analytic Review of the Neural Mechanisms of Vigilant Attention." *Psychological Bulletin* 139 (4): 870–900. https://doi.org/10.1037/a0030694.

Laursen, Thomas M, Trine Munk-Olsen, and Mogens Vestergaard. 2012. "Life Expectancy and Cardiovascular Mortality in Persons with Schizophrenia." *Current Opinion in Psychiatry* 25 (2): 83–88. https://doi.org/10.1097/YCO.0b013e32835035ca.

Laursen, Thomas Munk, Merete Nordentoft, and Preben Bo Mortensen. 2014. "Excess Early Mortality in Schizophrenia." *Annual Review of Clinical Psychology*. https://doi.org/10.1146/annurev-clinpsy-032813-153657.

Lavoie, Joëlle, Patricia Gassó Astorga, Hadar Segal-Gavish, Yee Wen Candace Wu, Youjin Chung, Nicola G. Cascella, Akira Sawa, and Koko Ishizuka. 2017. "The Olfactory Neural Epithelium As a Tool in Neuroscience." *Trends in Molecular Medicine*. Elsevier Ltd. https://doi.org/10.1016/j.molmed.2016.12.010.

Lavoie, Joelle, Akira Sawa, and Koko Ishizuka. 2017. "Application of Olfactory Tissue and Its Neural Progenitors to Schizophrenia and Psychiatric Research." *Current Opinion in Psychiatry* 30 (3): 176–83. https://doi.org/10.1097/YCO.0000000000000327.

Lee, Albert Muh Haur, Chong Guan Ng, Ong Hui Koh, Jesjeet Singh Gill, and Salina Abdul Aziz. 2018. "Metabolic Syndrome in First Episode Schizophrenia, Based on the National Mental Health Registry of Schizophrenia (NMHR) in a General Hospital in Malaysia: A 10-Year Retrospective Cohort Study." *International Journal of Environmental Research and Public Health* 15 (5). https://doi.org/10.3390/ijerph15050933.

Lee, M A, K Jayathilake, and H Y Meltzer. 1999. "A Comparison of the Effect of Clozapine with Typical Neuroleptics on Cognitive Function in Neuroleptic-Responsive Schizophrenia." *Schizophrenia Research* 37 (1): 1–11. https://doi.org/10.1016/s0920-9964(98)00145-5.

Leemput, J van de, J L Hess, S J Glatt, and M T Tsuang. 2016. "Genetics of Schizophrenia: Historical Insights and Prevailing Evidence." *Advances in Genetics* 96: 99–141. https://doi.org/10.1016/bs.adgen.2016.08.001.

Lesh, Tyler A, Tara A Niendam, Michael J Minzenberg, and Cameron S Carter. 2011. "Cognitive Control Deficits in Schizophrenia: Mechanisms and Meaning." *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology* 36 (1): 316–38. https://doi.org/10.1038/npp.2010.156.

Leucht, Stefan, Magdolna Tardy, Katja Komossa, Stephan Heres, Werner Kissling, Georgia Salanti, and John M Davis. 2012. "Antipsychotic Drugs versus Placebo for Relapse Prevention in Schizophrenia: A Systematic Review and Meta-Analysis." *Lancet (London, England)* 379 (9831): 2063–71. https://doi.org/10.1016/S0140-6736(12)60239-6.

Leung, Cheuk T, Pierre A Coulombe, and Randall R Reed. 2007. "Contribution of Olfactory Neural Stem Cells to Tissue Maintenance and Regeneration." *Nature Neuroscience* 10 (6): 720–26. https://doi.org/10.1038/nn1882.

Leweke, F M, D Piomelli, F Pahlisch, D Muhl, C W Gerth, C Hoyer, J Klosterkötter, M Hellmich, and D Koethe. 2012. "Cannabidiol Enhances Anandamide Signaling and Alleviates Psychotic Symptoms of Schizophrenia." *Translational Psychiatry* 2 (3): e94. https://doi.org/10.1038/tp.2012.15.

Linszen, D H, P M Dingemans, and M E Lenior. 1994. "Cannabis Abuse and the Course of Recent-Onset Schizophrenic Disorders." *Archives of General Psychiatry* 51 (4): 273–79. https://doi.org/10.1001/archpsyc.1994.03950040017002.

López-Giménez, Juan F, and Javier González-Maeso. 2018. "Hallucinogens and Serotonin 5-HT(2A) Receptor-Mediated Signaling Pathways." *Current Topics in Behavioral Neurosciences* 36: 45–73. https://doi.org/10.1007/7854_2017_478.

Lorenzetti, Valentina, Nadia Solowij, and Murat Yücel. 2016. "The Role of Cannabinoids in Neuroanatomic Alterations in Cannabis Users." *Biological Psychiatry* 79 (7): e17-31. https://doi.org/10.1016/j.biopsych.2015.11.013.

Lu, Hui-Chen, and Ken Mackie. 2016. "An Introduction to the Endogenous Cannabinoid System." *Biological Psychiatry* 79 (7): 516–25. https://doi.org/10.1016/j.biopsych.2015.07.028.

Mackay-Sim, Alan. 2012. "Concise Review: Patient-Derived Olfactory Stem Cells: New Models for Brain Diseases." *Stem Cells (Dayton, Ohio)* 30 (11): 2361–65. https://doi.org/10.1002/stem.1220.

Magalhães, Carolina Peixoto, Manuela Figueiroa Lyra de Freitas, Maria Inês Nogueira, Renata Cristinny de Farias Campina, Luiz Fernando Takase, Sandra Lopes de Souza, and Raul Manhães de Castro. 2010. "Modulatory Role of Serotonin on Feeding Behavior." *Nutritional Neuroscience* 13 (6): 246–55. https://doi.org/10.1179/147683010X12611460764723.

Malone, Daniel T, Matthew N Hill, and Tiziana Rubino. 2010. "Adolescent Cannabis Use and Psychosis: Epidemiology and Neurodevelopmental Models." *British Journal of Pharmacology* 160 (3): 511–22. https://doi.org/10.1111/j.1476-5381.2010.00721.x.

Mané, Anna, Daniel Bergé, Maria Jose Penzol, Mara Parellada, Miquel Bioque, Antonio Lobo, Ana González-Pinto, et al. 2017. "Cannabis Use, COMT, BDNF and Age at First-Episode Psychosis." *Psychiatry Research* 250 (April): 38–43. https://doi.org/10.1016/j.psychres.2017.01.045.

Mané, Anna, Miguel Fernández-Expósito, Daniel Bergé, Laura Gómez-Pérez, Agnés Sabaté, Alba Toll, Laura Diaz, Cristobal Diez-Aja, and Victor Perez. 2015. "Relationship between Cannabis and Psychosis: Reasons for Use and Associated Clinical Variables." *Psychiatry Research* 229 (1–2): 70–74. https://doi.org/10.1016/j.psychres.2015.07.070.

Marconi, Arianna, Marta Di Forti, Cathryn M Lewis, Robin M Murray, and Evangelos Vassos. 2016. "Meta-Analysis of the Association Between the Level of Cannabis Use and Risk of Psychosis." *Schizophrenia Bulletin* 42 (5): 1262–69. https://doi.org/10.1093/schbul/sbw003.

Marder, Stephen R, and Tyrone D Cannon. 2019. "Schizophrenia." *The New England Journal of Medicine* 381 (18): 1753–61. https://doi.org/10.1056/NEJMra1808803.

Marder, Stephen R, and Silvana Galderisi. 2017. "The Current Conceptualization of Negative Symptoms in Schizophrenia." *World Psychiatry : Official Journal of the World Psychiatric Association (WPA)* 16 (1): 14–24. https://doi.org/10.1002/wps.20385.

Marshall, Charles Taylor, Zhanfang Guo, Chengliang Lu, Kathleen M Klueber, Abdelnaby Khalyfa, Nigel G F Cooper, and Fred J Roisen. 2005. "Human Adult Olfactory Neuroepithelial Derived Progenitors Retain Telomerase Activity and Lack Apoptotic Activity." *Brain Research* 1045 (1–2): 45–56. https://doi.org/10.1016/j.brainres.2005.03.041.

Marzo, V Di, A Fontana, H Cadas, S Schinelli, G Cimino, J C Schwartz, and D Piomelli. 1994. "Formation and Inactivation of Endogenous Cannabinoid Anandamide in Central Neurons." *Nature* 372 (6507):

686–91. https://doi.org/10.1038/372686a0.

Matigian, Nicholas, Greger Abrahamsen, Ratneswary Sutharsan, Anthony L Cook, Alejandra M Vitale, Amanda Nouwens, Bernadette Bellette, et al. 2010. “Disease-Specific, Neurosphere-Derived Cells as Models for Brain Disorders.” *Disease Models & Mechanisms* 3 (11–12): 785–98. https://doi.org/10.1242/dmm.005447.

McGrath, John, Sukanta Saha, David Chant, and Joy Welham. 2008. “Schizophrenia: A Concise Overview of Incidence, Prevalence, and Mortality.” *Epidemiologic Reviews* 30: 67–76. https://doi.org/10.1093/epirev/mxn001.

Mechoulam, R, S Ben-Shabat, L Hanus, M Ligumsky, N E Kaminski, A R Schatz, A Gopher, S Almog, B R Martin, and D R Compton. 1995. “Identification of an Endogenous 2-Monoglyceride, Present in Canine Gut, That Binds to Cannabinoid Receptors.” *Biochemical Pharmacology* 50 (1): 83–90. https://doi.org/10.1016/0006-2952(95)00109-d.

Meltzer, H Y, and B W Massey. 2011. “The Role of Serotonin Receptors in the Action of Atypical Antipsychotic Drugs.” *Current Opinion in Pharmacology* 11 (1): 59–67. https://doi.org/10.1016/j.coph.2011.02.007.

Meltzer, H Y, and S R McGurk. 1999. “The Effects of Clozapine, Risperidone, and Olanzapine on Cognitive Function in Schizophrenia.” *Schizophrenia Bulletin* 25 (2): 233–55. https://doi.org/10.1093/oxfordjournals.schbul.a033376.

Menniti, Frank S, Craig W Lindsley, P Jeffrey Conn, Jayvardhan Pandit, Panayiotis Zagouras, and Robert A Volkmann. 2013. “Allosteric Modulators for the Treatment of Schizophrenia: Targeting Glutamatergic Networks.” *Current Topics in Medicinal Chemistry* 13 (1): 26–54.

Merwe, C van der, R Passchier, M Mufford, R Ramesar, S Dalvie, and D J Stein. 2019. “Polygenic Risk for Schizophrenia and Associated Brain Structural Changes: A Systematic Review.” *Comprehensive Psychiatry* 88 (January): 77–82. https://doi.org/10.1016/j.comppsych.2018.11.014.

Mesholam-Gately, Raquelle I, Anthony J Giuliano, Kirsten P Goff, Stephen V Faraone, and Larry J Seidman. 2009. “Neurocognition in First-Episode Schizophrenia: A Meta-Analytic Review.” *Neuropsychology* 23 (3): 315–36. https://doi.org/10.1037/a0014708.

Mittal, Vijay A, Wendy Hasenkamp, Michael Sanfilipo, Susan Wieland, Burton Angrist, John Rotrosen, and Erica J Duncan. 2007. “Relation of Neurological Soft Signs to Psychiatric Symptoms in Schizophrenia.” *Schizophrenia Research* 94 (1–3): 37–44. https://doi.org/10.1016/j.schres.2007.04.017.

Miyamoto, Seiya, G E Duncan, C E Marx, and J A Lieberman. 2005. “Treatments for Schizophrenia: A Critical Review of Pharmacology and Mechanisms of Action of Antipsychotic Drugs.” *Molecular Psychiatry* 10 (1): 79–104. https://doi.org/10.1038/sj.mp.4001556.

Miyamoto, Seiya, Anthony S LaMantia, Gary E Duncan, Patrick Sullivan, John H Gilmore, and Jeffrey A Lieberman. 2003. “Recent Advances in the Neurobiology of Schizophrenia.” *Molecular Interventions* 3 (1): 27–39. https://doi.org/10.1124/mi.3.1.27.

Miyazaki, Katsuhiko, Kayoko W Miyazaki, and Kenji Doya. 2012. “The Role of Serotonin in the Regulation

of Patience and Impulsivity." *Molecular Neurobiology* 45 (2): 213–24. https://doi.org/10.1007/s12035-012-8232-6.

Mizrahi, Romina, Jeremy J Watts, and Kuei Y Tseng. 2017. "Mechanisms Contributing to Cognitive Deficits in Cannabis Users." *Neuropharmacology*. https://doi.org/10.1016/j.neuropharm.2017.04.018.

Momen, Natalie C, Oleguer Plana-Ripoll, Esben Agerbo, Michael E Benros, Anders D Børglum, Maria K Christensen, Søren Dalsgaard, et al. 2020. "Association between Mental Disorders and Subsequent Medical Conditions." *The New England Journal of Medicine* 382 (18): 1721–31. https://doi.org/10.1056/NEJMoa1915784.

Moore, S, D Shiers, B Daly, A J Mitchell, and F Gaughran. 2015. "Promoting Physical Health for People with Schizophrenia by Reducing Disparities in Medical and Dental Care." *Acta Psychiatrica Scandinavica* 132 (2): 109–21. https://doi.org/10.1111/acps.12431.

Moore, Theresa H M, Stanley Zammit, Anne Lingford-Hughes, Thomas R E Barnes, Peter B Jones, Margaret Burke, and Glyn Lewis. 2007. "Cannabis Use and Risk of Psychotic or Affective Mental Health Outcomes: A Systematic Review." *Lancet (London, England)* 370 (9584): 319–28. https://doi.org/10.1016/S0140-6736(07)61162-3.

Mor, Eyal, Shin-Ichi Kano, Carlo Colantuoni, Akira Sawa, Ruth Navon, and Noam Shomron. 2013. "MicroRNA-382 Expression Is Elevated in the Olfactory Neuroepithelium of Schizophrenia Patients." *Neurobiology of Disease* 55 (July): 1–10. https://doi.org/10.1016/j.nbd.2013.03.011.

Moreno-Küstner, Berta, Carlos Martín, and Loly Pastor. 2018. "Prevalence of Psychotic Disorders and Its Association with Methodological Issues. A Systematic Review and Meta-Analyses." *PloS One* 13 (4): e0195687. https://doi.org/10.1371/journal.pone.0195687.

Mounteney, Jane, Paul Griffiths, Roumen Sedefov, Andre Noor, Julián Vicente, and Roland Simon. 2016. "The Drug Situation in Europe: An Overview of Data Available on Illicit Drugs and New Psychoactive Substances from European Monitoring in 2015." *Addiction (Abingdon, England)*. https://doi.org/10.1111/add.13056.

Mucci, Armida, Eleonora Merlotti, Alp Üçok, André Aleman, and Silvana Galderisi. 2017. "Primary and Persistent Negative Symptoms: Concepts, Assessments and Neurobiological Bases." *Schizophrenia Research* 186 (August): 19–28. https://doi.org/10.1016/j.schres.2016.05.014.

Muguruza, Carolina, Marko Lehtonen, Niina Aaltonen, Benito Morentin, J Javier Meana, and Luis F Callado. 2013. "Quantification of Endocannabinoids in Postmortem Brain of Schizophrenic Subjects." *Schizophrenia Research* 148 (1–3): 145–50. https://doi.org/10.1016/j.schres.2013.06.013.

Muguruza, Carolina, Jose L Moreno, Adrienne Umali, Luis F Callado, J Javier Meana, and Javier Gonzalez-Maeso. 2013. "Dysregulated 5-HT(2A) Receptor Binding in Postmortem Frontal Cortex of Schizophrenic Subjects." *European Neuropsychopharmacology : The Journal of the European College of Neuropsychopharmacology* 23 (8): 852–64. https://doi.org/10.1016/j.euroneuro.2012.10.006.

Muguruza, Carolina, Benito Morentin, J Javier Meana, Stephen Ph Alexander, and Luis F Callado. 2019.

"Endocannabinoid System Imbalance in the Postmortem Prefrontal Cortex of Subjects with Schizophrenia." *Journal of Psychopharmacology (Oxford, England)* 33 (9): 1132–40. https://doi.org/10.1177/0269881119857205.

Müller, Christian P, and Barry L Jacobs, eds. 2010. *Handbook of the Behavioral Neurobiology of Serotonin. Handbook of the Behavioral Neurobiology of Serotonin.* Müller, Christian P.: christian.muller@kcl.ac.uk: Elsevier Academic Press.

Muñoz-Estrada, Jesús, Gloria Benítez-King, Carlos Berlanga, and Isaura Meza. 2015. "Altered Subcellular Distribution of the 75-KDa DISC1 Isoform, CAMP Accumulation, and Decreased Neuronal Migration in Schizophrenia and Bipolar Disorder: Implications for Neurodevelopment." *CNS Neuroscience and Therapeutics* 21 (5): 446–53. https://doi.org/10.1111/cns.12377.

Muñoz-estrada, Jesús, Alejandra Lora-castellanos, Isaura Meza, Salvador Alarcón, and Gloria Benítez-king. 2018. "Primary Cilia Formation Is Diminished in Schizophrenia and Bipolar Disorder : A Possible Marker for These Psychiatric Diseases." *Schizophrenia Research* 195: 412–20. https://doi.org/10.1016/j.schres.2017.08.055.

Navarrete, Francisco, María Salud García-Gutiérrez, Rosa Jurado-Barba, Gabriel Rubio, Ani Gasparyan, Amaya Austrich-Olivares, and Jorge Manzanares. 2020. "Endocannabinoid System Components as Potential Biomarkers in Psychiatry." *Frontiers in Psychiatry* 11: 315. https://doi.org/10.3389/fpsyt.2020.00315.

Neelam, Kishen, Deepak Garg, and Max Marshall. 2011. "A Systematic Review and Meta-Analysis of Neurological Soft Signs in Relatives of People with Schizophrenia." *BMC Psychiatry* 11 (August): 139. https://doi.org/10.1186/1471-244X-11-139.

Newell, Kelly A, Chao Deng, and Xu-Feng Huang. 2006. "Increased Cannabinoid Receptor Density in the Posterior Cingulate Cortex in Schizophrenia." *Experimental Brain Research* 172 (4): 556–60. https://doi.org/10.1007/s00221-006-0503-x.

Nguyen, An D, Martha E Shenton, and James J Levitt. 2010. "Olfactory Dysfunction in Schizophrenia: A Review of Neuroanatomy and Psychophysiological Measurements." *Harvard Review of Psychiatry* 18 (5): 279–92. https://doi.org/10.3109/10673229.2010.511060.

Oakley, Padraig, Steve Kisely, Amanda Baxter, Meredith Harris, Jocelyne Desoe, Alyona Dziouba, and Dan Siskind. 2018. "Increased Mortality among People with Schizophrenia and Other Non-Affective Psychotic Disorders in the Community: A Systematic Review and Meta-Analysis." *Journal of Psychiatric Research* 102 (July): 245–53. https://doi.org/10.1016/j.jpsychires.2018.04.019.

Olfson, Mark, Tobias Gerhard, Cecilia Huang, Stephen Crystal, and T Scott Stroup. 2015. "Premature Mortality Among Adults With Schizophrenia in the United States." *JAMA Psychiatry* 72 (12): 1172–81. https://doi.org/10.1001/jamapsychiatry.2015.1737.

Ortiz-López, Leonardo, Jorge Julio González-Olvera, Nelly Maritza Vega-Rivera, Maria García-Anaya, Ana Karen Carapia-Hernández, Julio César Velázquez-Escobar, and Gerardo Bernabé Ramírez-Rodríguez. 2017. "Human Neural Stem/Progenitor Cells Derived from the Olfactory Epithelium Express the TrkB Receptor and Migrate in Response to BDNF." *Neuroscience* 355 (July): 84–100.

https://doi.org/10.1016/j.neuroscience.2017.04.047.

Parrish, Jason C, and David E Nichols. 2006. “Serotonin 5-HT(2A) Receptor Activation Induces 2-Arachidonoylglycerol Release through a Phospholipase c-Dependent Mechanism.” *Journal of Neurochemistry* 99 (4): 1164–75. https://doi.org/10.1111/j.1471-4159.2006.04173.x.

Penadés, Rafael, Nicolas Franck, Laura González-Vallespí, and Marie Dekerle. 2019. “Neuroimaging Studies of Cognitive Function in Schizophrenia.” *Advances in Experimental Medicine and Biology* 1118: 117–34. https://doi.org/10.1007/978-3-030-05542-4_6.

Pertwee, Roger. 2014. *Handbook of Cannabis*. Oxford: Oxford University Press. https://doi.org/10.1093/acprof:oso/9780199662685.001.0001.

Petersen, Stine Mai, Nanna Gilliam Toftdahl, Merete Nordentoft, and Carsten Hjorthoj. 2019. “Schizophrenia Is Associated with Increased Risk of Subsequent Substance Abuse Diagnosis: A Nation-Wide Population-Based Register Study.” *Addiction (Abingdon, England)*, July. https://doi.org/10.1111/add.14746.

Plana-Ripoll, Oleguer, Carsten Bøcker Pedersen, Yan Holtz, Michael E Benros, Søren Dalsgaard, Peter de Jonge, Chun Chieh Fan, et al. 2019. “Exploring Comorbidity Within Mental Disorders Among a Danish National Population.” *JAMA Psychiatry* 76 (3): 259–70. https://doi.org/10.1001/jamapsychiatry.2018.3658.

Pollak Dorocic, Iskra, Daniel Fürth, Yang Xuan, Yvonne Johansson, Laura Pozzi, Gilad Silberberg, Marie Carlén, and Konstantinos Meletis. 2014. “A Whole-Brain Atlas of Inputs to Serotonergic Neurons of the Dorsal and Median Raphe Nuclei.” *Neuron* 83 (3): 663–78. https://doi.org/10.1016/j.neuron.2014.07.002.

Popovic, D, A Benabarre, J M Crespo, J M Goikolea, A González-Pinto, L Gutiérrez-Rojas, J M Montes, and E Vieta. 2014. “Risk Factors for Suicide in Schizophrenia: Systematic Review and Clinical Recommendations.” *Acta Psychiatrica Scandinavica* 130 (6): 418–26. https://doi.org/10.1111/acps.12332.

Portas, C M, B Bjorvatn, and R Ursin. 2000. “Serotonin and the Sleep/Wake Cycle: Special Emphasis on Microdialysis Studies.” *Progress in Neurobiology* 60 (1): 13–35. https://doi.org/10.1016/s0301-0082(98)00097-5.

Potkin, Steven G, Fabio Macciardi, Guia Guffanti, James H Fallon, Qi Wang, Jessica A Turner, Anita Lakatos, et al. 2010. “Identifying Gene Regulatory Networks in Schizophrenia.” *NeuroImage* 53 (3): 839–47. https://doi.org/10.1016/j.neuroimage.2010.06.036.

Potvin, Stéphane, and Mohamed Ben Amar. 2008. “Review: Cannabis Use Increases the Risk of Psychotic Outcomes.” *Evidence-Based Mental Health* 11 (1): 28. https://doi.org/10.1136/ebmh.11.1.28.

Power, R A, K J H Verweij, M Zuhair, G W Montgomery, A K Henders, A C Heath, P A F Madden, S E Medland, N R Wray, and N G Martin. 2014. “Genetic Predisposition to Schizophrenia Associated with Increased Use of Cannabis.” *Molecular Psychiatry* 19 (11): 1201–4. https://doi.org/10.1038/mp.2014.51.

Priestley, Richard, Michelle Glass, and David Kendall. 2017. “Chapter Six - Functional Selectivity at

Cannabinoid Receptors." In *Cannabinoid Pharmacology*, edited by David Kendall and Stephen P H B T - Advances in Pharmacology Alexander, 80:207–21. Academic Press. https://doi.org/https://doi.org/10.1016/bs.apha.2017.03.005.

Purcell, Shaun M, Jennifer L Moran, Menachem Fromer, Douglas Ruderfer, Nadia Solovieff, Panos Roussos, Colm O'Dushlaine, et al. 2014. "A Polygenic Burden of Rare Disruptive Mutations in Schizophrenia." *Nature* 506 (7487): 185–90. https://doi.org/10.1038/nature12975.

Purcell, Shaun M, Naomi R Wray, Jennifer L Stone, Peter M Visscher, Michael C O'Donovan, Patrick F Sullivan, and Pamela Sklar. 2009. "Common Polygenic Variation Contributes to Risk of Schizophrenia and Bipolar Disorder." *Nature* 460 (7256): 748–52. https://doi.org/10.1038/nature08185.

Quednow, Boris B, Ingo Frommann, Julia Berning, Kai-Uwe Kühn, Wolfgang Maier, and Michael Wagner. 2008. "Impaired Sensorimotor Gating of the Acoustic Startle Response in the Prodrome of Schizophrenia." *Biological Psychiatry* 64 (9): 766–73. https://doi.org/10.1016/j.biopsych.2008.04.019.

Rabinowitz, Jonathan, Stephen Z Levine, George Garibaldi, Dragana Bugarski-Kirola, Carmen Galani Berardo, and Shitij Kapur. 2012. "Negative Symptoms Have Greater Impact on Functioning than Positive Symptoms in Schizophrenia: Analysis of CATIE Data." *Schizophrenia Research* 137 (1–3): 147–50. https://doi.org/10.1016/j.schres.2012.01.015.

Radhakrishnan, Rajiv, Muzaffer Kaser, and Sinan Guloksuz. 2017. "The Link Between the Immune System, Environment, and Psychosis." *Schizophrenia Bulletin* 43 (4): 693–97. https://doi.org/10.1093/schbul/sbx057.

Radhakrishnan, Rajiv, David Matuskey, Nabeel Nabulsi, Edward Gaiser, Jean-Dominique Gallezot, Shannan Henry, Beata Planeta, et al. 2020. "In Vivo 5-HT(6) and 5-HT(2A) Receptor Availability in Antipsychotic Treated Schizophrenia Patients vs. Unmedicated Healthy Humans Measured with [(11)C]GSK215083 PET." *Psychiatry Research. Neuroimaging* 295 (January): 111007. https://doi.org/10.1016/j.pscychresns.2019.111007.

Radua, Joaquim, Valentina Ramella-Cravaro, John P A Ioannidis, Abraham Reichenberg, Nacharin Phiphopthatsanee, Taha Amir, Hyi Yenn Thoo, et al. 2018. "What Causes Psychosis? An Umbrella Review of Risk and Protective Factors." *World Psychiatry : Official Journal of the World Psychiatric Association (WPA)* 17 (1): 49–66. https://doi.org/10.1002/wps.20490.

Rammos, Alexandros, Lara A Neira Gonzalez, Daniel R Weinberger, Kevin J Mitchell, and Kristin K Nicodemus. 2019. "The Role of Polygenic Risk Score Gene-Set Analysis in the Context of the Omnigenic Model of Schizophrenia." *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology* 44 (9): 1562–69. https://doi.org/10.1038/s41386-019-0410-z.

Ranganathan, Mohini, Jose Cortes-Briones, Rajiv Radhakrishnan, Halle Thurnauer, Beata Planeta, Patrick Skosnik, Hong Gao, et al. 2016. "Reduced Brain Cannabinoid Receptor Availability in Schizophrenia." *Biological Psychiatry* 79 (12): 997–1005.

https://doi.org/10.1016/j.biopsych.2015.08.021.

Rasmussen, Hans, David Erritzoe, Rune Andersen, Bjorn H Ebdrup, Bodil Aggernaes, Bob Oranje, Jan Kalbitzer, et al. 2010. "Decreased Frontal Serotonin2A Receptor Binding in Antipsychotic-Naive Patients with First-Episode Schizophrenia." *Archives of General Psychiatry* 67 (1): 9–16. https://doi.org/10.1001/archgenpsychiatry.2009.176.

Rioux, Lise, Edward Isaac Gelber, Leila Parand, Hala Altaf Kazi, Joannie Yeh, Rebecca Wintering, Warren Bilker, and Steven Edward Arnold. 2005. "Characterization of Olfactory Bulb Glomeruli in Schizophrenia." *Schizophrenia Research* 77 (2–3): 229–39. https://doi.org/10.1016/j.schres.2005.04.022.

Ripke, Stephan, Colm O'Dushlaine, Kimberly Chambert, Jennifer L Moran, Anna K Kähler, Susanne Akterin, Sarah E Bergen, et al. 2013. "Genome-Wide Association Analysis Identifies 13 New Risk Loci for Schizophrenia." *Nature Genetics* 45 (10): 1150–59. https://doi.org/10.1038/ng.2742.

Rodríguez-Sánchez, José Manuel, Esther Setién-Suero, Paula Suárez-Pinilla, Jaqueline Mayoral Van Son, Javier Vázquez-Bourgon, Patxi Gil López, Benedicto Crespo-Facorro, and Rosa Ayesa-Arriola. 2020. "Ten-Year Course of Cognition in First-Episode Non-Affective Psychosis Patients: PAFIP Cohort." *Psychological Medicine*, July, 1–10. https://doi.org/10.1017/S0033291720002408.

Rosenberg, Monica D, Emily S Finn, Dustin Scheinost, Xenophon Papademetris, Xilin Shen, R Todd Constable, and Marvin M Chun. 2016. "A Neuromarker of Sustained Attention from Whole-Brain Functional Connectivity." *Nature Neuroscience* 19 (1): 165–71. https://doi.org/10.1038/nn.4179.

Roth, Tania L, Farah D Lubin, Monsheel Sodhi, and Joel E Kleinman. 2009. "Epigenetic Mechanisms in Schizophrenia." *Biochimica et Biophysica Acta* 1790 (9): 869–77. https://doi.org/10.1016/j.bbagen.2009.06.009.

Ruiz-Veguilla, Miguel, Luis F Callado, and Maite Ferrin. 2012. "Neurological Soft Signs in Patients with Psychosis and Cannabis Abuse: A Systematic Review and Meta-Analysis of Paradox." *Current Pharmaceutical Design* 18 (32): 5156–64. https://doi.org/10.2174/138161212802884753.

Saha, Sukanta, David Chant, and John McGrath. 2007. "A Systematic Review of Mortality in Schizophrenia: Is the Differential Mortality Gap Worsening over Time?" *Archives of General Psychiatry* 64 (10): 1123–31. https://doi.org/10.1001/archpsyc.64.10.1123.

Sakurai, Hitoshi, Robert R Bies, Scott T Stroup, Richard S E Keefe, Tarek K Rajji, Takefumi Suzuki, David C Mamo, et al. 2013. "Dopamine D2 Receptor Occupancy and Cognition in Schizophrenia: Analysis of the CATIE Data." *Schizophrenia Bulletin* 39 (3): 564–74. https://doi.org/10.1093/schbul/sbr189.

Salomon, Joshua A, Theo Vos, Daniel R Hogan, Michael Gagnon, Mohsen Naghavi, Ali Mokdad, Nazma Begum, et al. 2012. "Common Values in Assessing Health Outcomes from Disease and Injury: Disability Weights Measurement Study for the Global Burden of Disease Study 2010." *Lancet (London, England)* 380 (9859): 2129–43. https://doi.org/10.1016/S0140-6736(12)61680-8.

Santini, Martin A, Cecilia Ratner, Susana Aznar, Anders B Klein, Gitte M Knudsen, and Jens D Mikkelsen. 2013. "Enhanced Prefrontal Serotonin 2A Receptor Signaling in the Subchronic Phencyclidine Mouse Model of Schizophrenia." *Journal of Neuroscience Research* 91 (5): 634–41.

https://doi.org/10.1002/jnr.23198.

Santos, Rafael Guimarães Dos, José Alexandre Crippa, Flávia de Lima Osório, Juliana M Rocha, Giordano Novak Rossi, Camila Marchioni, Maria Eugênia Costa Queiroz, Gabriela O Silveira, Mauricio Yonamine, and Jaime Eduardo Cecilio Hallak. 2018. "Possible Interactions Between 5-HT2A Receptors and the Endocannabinoid System in Humans: Preliminary Evidence of Interactive Effects of Ayahuasca and Endocannabinoids in a Healthy Human Subject." *Journal of Clinical Psychopharmacology*. United States. https://doi.org/10.1097/JCP.0000000000000973.

Savulich, George, Gisela Mezquida, Samuel Atkinson, Miguel Bernardo, and Emilio Fernandez-Egea. 2018. "A Case Study of Clozapine and Cognition: Friend or Foe?" *Journal of Clinical Psychopharmacology*. United States. https://doi.org/10.1097/JCP.0000000000000847.

Sawa, Akira, and Nicola G. Cascella. 2009. "Peripheral Olfactory System for Clinical and Basic Psychiatry: A Promising Entry Point to the Mystery of Brain Mechanism and Biomarker Identification in Schizophrenia." *American Journal of Psychiatry*. https://doi.org/10.1176/appi.ajp.2008.08111702.

Schnell, Thomas, Dagmar Koethe, Anna Krasnianski, Stefanie Gairing, Knut Schnell, Jörg Daumann, and Euphrosyne Gouzoulis-Mayfrank. 2014. "Ziprasidone versus Clozapine in the Treatment of Dually Diagnosed (DD) Patients with Schizophrenia and Cannabis Use Disorders: A Randomized Study." *The American Journal on Addictions* 23 (3): 308–12. https://doi.org/10.1111/j.1521-0391.2014.12126.x.

Schoenbaum, Michael, Jason M Sutherland, Andre Chappel, Susan Azrin, Amy B Goldstein, Agnes Rupp, and Robert K Heinssen. 2017. "Twelve-Month Health Care Use and Mortality in Commercially Insured Young People With Incident Psychosis in the United States." *Schizophrenia Bulletin* 43 (6): 1262–72. https://doi.org/10.1093/schbul/sbx009.

Schreiner, Amy M, and Michael E Dunn. 2012. "Residual Effects of Cannabis Use on Neurocognitive Performance after Prolonged Abstinence: A Meta-Analysis." *Experimental and Clinical Psychopharmacology* 20 (5): 420–29. https://doi.org/10.1037/a0029117.

Selvaraj, Sudhakar, Danilo Arnone, Alessandra Cappai, and Oliver Howes. 2014. "Alterations in the Serotonin System in Schizophrenia: A Systematic Review and Meta-Analysis of Postmortem and Molecular Imaging Studies." *Neuroscience and Biobehavioral Reviews* 45 (September): 233–45. https://doi.org/10.1016/j.neubiorev.2014.06.005.

Sheffield, Julia M, and Deanna M Barch. 2016. "Cognition and Resting-State Functional Connectivity in Schizophrenia." *Neuroscience and Biobehavioral Reviews* 61 (February): 108–20. https://doi.org/10.1016/j.neubiorev.2015.12.007.

Sherif, Mohamed, Rajiv Radhakrishnan, Deepak Cyril D'Souza, and Mohini Ranganathan. 2016. "Human Laboratory Studies on Cannabinoids and Psychosis." *Biological Psychiatry* 79 (7): 526–38. https://doi.org/10.1016/j.biopsych.2016.01.011.

Smutzer, G, V M Lee, J Q Trojanowski, and S E Arnold. 1998. "Human Olfactory Mucosa in Schizophrenia." *The Annals of Otology, Rhinology, and Laryngology* 107 (4): 349–55. https://doi.org/10.1177/000348949810700415.

Solmi, Marco, Joseph Firth, Alessandro Miola, Michele Fornaro, Elisabetta Frison, Paolo Fusar-Poli, Elena Dragioti, et al. 2020. “Disparities in Cancer Screening in People with Mental Illness across the World versus the General Population: Prevalence and Comparative Meta-Analysis Including 4 717 839 People.” *The Lancet. Psychiatry* 7 (1): 52–63. https://doi.org/10.1016/S2215-0366(19)30414-6.

Solmi, Marco, Andrea Murru, Isabella Pacchiarotti, Juan Undurraga, Nicola Veronese, Michele Fornaro, Brendon Stubbs, et al. 2017. “Safety, Tolerability, and Risks Associated with First- and Second-Generation Antipsychotics: A State-of-the-Art Clinical Review.” *Therapeutics and Clinical Risk Management* 13: 757–77. https://doi.org/10.2147/TCRM.S117321.

Solowij, Nadia, and Robert Battisti. 2008. “The Chronic Effects of Cannabis on Memory in Humans: A Review.” *Current Drug Abuse Reviews* 1 (1): 81–98.

Soria-Gomez, Edgar, Luigi Bellocchio, and Giovanni Marsicano. 2014. “New Insights on Food Intake Control by Olfactory Processes: The Emerging Role of the Endocannabinoid System.” *Molecular and Cellular Endocrinology* 397 (1–2): 59–66. https://doi.org/10.1016/j.mce.2014.09.023.

Sponheim, S R, R E Jung, L J Seidman, R I Mesholam-Gately, D S Manoach, D S O’Leary, B C Ho, N C Andreasen, J Lauriello, and S C Schulz. 2010. “Cognitive Deficits in Recent-Onset and Chronic Schizophrenia.” *Journal of Psychiatric Research* 44 (7): 421–28. https://doi.org/10.1016/j.jpsychires.2009.09.010.

Stępnicki, Piotr, Magda Kondej, and Agnieszka A Kaczor. 2018. “Current Concepts and Treatments of Schizophrenia.” *Molecules (Basel, Switzerland)* 23 (8). https://doi.org/10.3390/molecules23082087.

Stone, J M, H L Fisher, B Major, B Chisholm, J Woolley, J Lawrence, N Rahaman, et al. 2014. “Cannabis Use and First-Episode Psychosis: Relationship with Manic and Psychotic Symptoms, and with Age at Presentation.” *Psychological Medicine* 44 (3): 499–506. https://doi.org/10.1017/S0033291713000883.

Tang, Sephora M, Aylar Ansarian, and Darren B Courtney. 2017. “Clozapine Treatment and Cannabis Use in Adolescents with Psychotic Disorders - A Retrospective Cohort Chart Review.” *Journal of the Canadian Academy of Child and Adolescent Psychiatry = Journal de l’Academie Canadienne de Psychiatrie de l’enfant et de l’adolescent* 26 (1): 51–58.

Tanskanen, A, J Tiihonen, and H Taipale. 2018. “Mortality in Schizophrenia: 30-Year Nationwide Follow-up Study.” *Acta Psychiatrica Scandinavica* 138 (6): 492–99. https://doi.org/10.1111/acps.12913.

Tee, Jing Yang, Ratneswary Sutharsan, Yongjun Fan, and Alan Mackay-Sim. 2017. “Cell Migration in Schizophrenia: Patient-Derived Cells Do Not Regulate Motility in Response to Extracellular Matrix.” *Molecular and Cellular Neurosciences* 80 (April): 111–22. https://doi.org/10.1016/j.mcn.2017.03.005.

Thieme, Ulrike, Gustav Schelling, Daniela Hauer, Robert Greif, Torsten Dame, Ruediger Paul Laubender, Werner Bernhard, Detlef Thieme, Patrizia Campolongo, and Lorenz Theiler. 2014. “Quantification of Anandamide and 2-Arachidonoylglycerol Plasma Levels to Examine Potential Influences of

Tetrahydrocannabinol Application on the Endocannabinoid System in Humans." *Drug Testing and Analysis* 6 (1–2): 17–23. https://doi.org/10.1002/dta.1561.

Torrens, Marta, Paola C Rossi, Roser Martinez-Riera, Diana Martinez-Sanvisens, and Antoni Bulbena. 2012. "Psychiatric Co-Morbidity and Substance Use Disorders: Treatment in Parallel Systems or in One Integrated System?" *Substance Use & Misuse* 47 (8–9): 1005–14. https://doi.org/10.3109/10826084.2012.663296.

Trampush, Joey W, Todd Lencz, Pamela DeRosse, Majnu John, Juan A Gallego, Georgios Petrides, Youssef Hassoun, et al. 2015. "Relationship of Cognition to Clinical Response in First-Episode Schizophrenia Spectrum Disorders." *Schizophrenia Bulletin* 41 (6): 1237–47. https://doi.org/10.1093/schbul/sbv120.

Tregellas, Jason R, Jason Smucny, Josette G Harris, Ann Olincy, Keeran Maharajh, Eugene Kronberg, Lindsay C Eichman, Emma Lyons, and Robert Freedman. 2014. "Intrinsic Hippocampal Activity as a Biomarker for Cognition and Symptoms in Schizophrenia." *The American Journal of Psychiatry* 171 (5): 549–56. https://doi.org/10.1176/appi.ajp.2013.13070981.

Uchida, Hiroyuki, Hiroyoshi Takeuchi, Ariel Graff-Guerrero, Takefumi Suzuki, Koichiro Watanabe, and David C Mamo. 2011. "Dopamine D2 Receptor Occupancy and Clinical Effects: A Systematic Review and Pooled Analysis." *Journal of Clinical Psychopharmacology* 31 (4): 497–502. https://doi.org/10.1097/JCP.0b013e3182214aad.

Umathe, Sudhir N, Shyamshree S S Manna, and Nishant S Jain. 2011. "Involvement of Endocannabinoids in Antidepressant and Anti-Compulsive Effect of Fluoxetine in Mice." *Behavioural Brain Research* 223 (1): 125–34. https://doi.org/10.1016/j.bbr.2011.04.031.

Urigüen, Leyre, M Julia García-Fuster, Luis F Callado, Benito Morentin, Romano La Harpe, Vicent Casadó, Carmen Lluis, Rafael Franco, Jesús A García-Sevilla, and J Javier Meana. 2009. "Immunodensity and MRNA Expression of A2A Adenosine, D2 Dopamine, and CB1 Cannabinoid Receptors in Postmortem Frontal Cortex of Subjects with Schizophrenia: Effect of Antipsychotic Treatment." *Psychopharmacology* 206 (2): 313–24. https://doi.org/10.1007/s00213-009-1608-2.

Vaidya, Jatin G, Robert I Block, Daniel S O'Leary, Laura B Ponto, Mohamed M Ghoneim, and Antoine Bechara. 2012. "Effects of Chronic Marijuana Use on Brain Activity during Monetary Decision-Making." *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology* 37 (3): 618–29. https://doi.org/10.1038/npp.2011.227.

Varambally, Shivarama, Ganesan Venkatasubramanian, and Bangalore N Gangadhar. 2012. "Neurological Soft Signs in Schizophrenia - The Past, the Present and the Future." *Indian Journal of Psychiatry* 54 (1): 73–80. https://doi.org/10.4103/0019-5545.94653.

Vaucher, J, B J Keating, A M Lasserre, W Gan, D M Lyall, J Ward, D J Smith, et al. 2018. "Cannabis Use and Risk of Schizophrenia: A Mendelian Randomization Study." *Molecular Psychiatry* 23 (5): 1287–92. https://doi.org/10.1038/mp.2016.252.

Vázquez-Bourgon, Javier, Ignacio Mata, Roberto Roiz-Santiáñez, Rosa Ayesa-Arriola, Paula Suárez Pinilla, Diana Tordesillas-Gutiérrez, José Luis Vázquez-Barquero, and Benedicto Crespo-Facorro. 2014. "A

Disrupted-in-Schizophrenia 1 Gene Variant Is Associated with Clinical Symptomatology in Patients with First-Episode Psychosis." *Psychiatry Investigation* 11 (2): 186–91. https://doi.org/10.4306/pi.2014.11.2.186.

Ventura, Joseph, Kenneth L Subotnik, Michael J Gitlin, Denise Gretchen-Doorly, Arielle Ered, Kathleen F Villa, Gerhard S Hellemann, and Keith H Nuechterlein. 2015. "Negative Symptoms and Functioning during the First Year after a Recent Onset of Schizophrenia and 8 Years Later." *Schizophrenia Research* 161 (2–3): 407–13. https://doi.org/10.1016/j.schres.2014.10.043.

Verweij, Karin J H, Abdel Abdellaoui, Michel G Nivard, Alberto Sainz Cort, Lannie Ligthart, Harmen H M Draisma, Camelia C Minica, et al. 2017. "Short Communication: Genetic Association between Schizophrenia and Cannabis Use." *Drug and Alcohol Dependence* 171 (February): 117–21. https://doi.org/10.1016/j.drugalcdep.2016.09.022.

Vinals, Xavier, Estefania Moreno, Laurence Lanfumey, Arnau Cordomi, Antoni Pastor, Rafael de La Torre, Paola Gasperini, et al. 2015. "Cognitive Impairment Induced by Delta9-Tetrahydrocannabinol Occurs through Heteromers between Cannabinoid CB1 and Serotonin 5-HT2A Receptors." *PLoS Biology* 13 (7): e1002194. https://doi.org/10.1371/journal.pbio.1002194.

Volk, David W, Stephen M Eggan, Andrew G Horti, Dean F Wong, and David A Lewis. 2014. "Reciprocal Alterations in Cortical Cannabinoid Receptor 1 Binding Relative to Protein Immunoreactivity and Transcript Levels in Schizophrenia." *Schizophrenia Research* 159 (1): 124–29. https://doi.org/10.1016/j.schres.2014.07.017.

Volkow, Nora D, Ruben D Baler, Wilson M Compton, and Susan R B Weiss. 2014. "Adverse Health Effects of Marijuana Use." *The New England Journal of Medicine* 370 (23): 2219–27. https://doi.org/10.1056/NEJMra1402309.

Volkow, Nora D, James M Swanson, A Eden Evins, Lynn E DeLisi, Madeline H Meier, Raul Gonzalez, Michael A P Bloomfield, H Valerie Curran, and Ruben Baler. 2016. "Effects of Cannabis Use on Human Behavior, Including Cognition, Motivation, and Psychosis: A Review." *JAMA Psychiatry* 73 (3): 292–97. https://doi.org/10.1001/jamapsychiatry.2015.3278.

Walker, Elizabeth Reisinger, Robin E. McGee, and Benjamin G. Druss. 2015. "Mortality in Mental Disorders and Global Disease Burden Implications a Systematic Review and Meta-Analysis." *JAMA Psychiatry* 72 (4): 334–41. https://doi.org/10.1001/jamapsychiatry.2014.2502.

Walter, Carmen, Nerea Ferreirós, Philipp Bishay, Gerd Geisslinger, Irmgard Tegeder, and Jörn Lötsch. 2013. "Exogenous Delta^9-Tetrahydrocannabinol Influences Circulating Endogenous Cannabinoids in Humans." *Journal of Clinical Psychopharmacology* 33 (5): 699–705. https://doi.org/10.1097/JCP.0b013e3182984015.

Weiland, Barbara J, Rachel E Thayer, Brendan E Depue, Amithrupa Sabbineni, Angela D Bryan, and Kent E Hutchison. 2015. "Daily Marijuana Use Is Not Associated with Brain Morphometric Measures in Adolescents or Adults." *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience* 35 (4): 1505–12. https://doi.org/10.1523/JNEUROSCI.2946-14.2015.

Weinberg-Wolf, Hannah, Nicholas A Fagan, George M Anderson, Marios Tringides, Olga Dal Monte, and

Steve W C Chang. 2018. “The Effects of 5-Hydroxytryptophan on Attention and Central Serotonin Neurochemistry in the Rhesus Macaque.” *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology* 43 (7): 1589–98. https://doi.org/10.1038/s41386-017-0003-7.

Weinstein, Aviv, Abigail Livny, and Abraham Weizman. 2016. “Brain Imaging Studies on the Cognitive, Pharmacological and Neurobiological Effects of Cannabis in Humans: Evidence from Studies of Adult Users.” *Current Pharmaceutical Design* 22 (42): 6366–79. https://doi.org/10.2174/1381612822666160822151323.

Whitty, P, M Clarke, S Browne, O McTigue, M Kamali, L Feeney, A Lane, et al. 2003. “Prospective Evaluation of Neurological Soft Signs in First-Episode Schizophrenia in Relation to Psychopathology: State versus Trait Phenomena.” *Psychological Medicine* 33 (8): 1479–84. https://doi.org/10.1017/s0033291703008225.

Williamson, E M, and F J Evans. 2000. “Cannabinoids in Clinical Practice.” *Drugs* 60 (6): 1303–14. https://doi.org/10.2165/00003495-200060060-00005.

Wockner, L F, E P Noble, B R Lawford, R McD Young, C P Morris, V L J Whitehall, and J Voisey. 2014. “Genome-Wide DNA Methylation Analysis of Human Brain Tissue from Schizophrenia Patients.” *Translational Psychiatry* 4 (January): e339. https://doi.org/10.1038/tp.2013.111.

Wong, Dean F, Hiroto Kuwabara, Andrew G Horti, Vanessa Raymont, James Brasic, Maria Guevara, Weiguo Ye, et al. 2010. “Quantification of Cerebral Cannabinoid Receptors Subtype 1 (CB1) in Healthy Subjects and Schizophrenia by the Novel PET Radioligand [11C]OMAR.” *NeuroImage* 52 (4): 1505–13. https://doi.org/10.1016/j.neuroimage.2010.04.034.

Wright, A A, M Todorovic, J Tello-Velasquez, A J Rayfield, J A St John, and J A Ekberg. 2018. “Enhancing the Therapeutic Potential of Olfactory Ensheathing Cells in Spinal Cord Repair Using Neurotrophins.” *Cell Transplantation* 27 (6): 867–78. https://doi.org/10.1177/0963689718759472.

Yucel, Murat, Emre Bora, Dan I Lubman, Nadia Solowij, Warrick J Brewer, Sue M Cotton, Philippe Conus, et al. 2012. “The Impact of Cannabis Use on Cognitive Functioning in Patients with Schizophrenia: A Meta-Analysis of Existing Findings and New Data in a First-Episode Sample.” *Schizophrenia Bulletin* 38 (2): 316–30. https://doi.org/10.1093/schbul/sbq079.

Zalesky, Andrew, Nadia Solowij, Murat Yücel, Dan I Lubman, Michael Takagi, Ian H Harding, Valentina Lorenzetti, et al. 2012. “Effect of Long-Term Cannabis Use on Axonal Fibre Connectivity.” *Brain : A Journal of Neurology* 135 (Pt 7): 2245–55. https://doi.org/10.1093/brain/aws136.

Zavitsanou, Katerina, Therese Garrick, and Xu Feng Huang. 2004. “Selective Antagonist [3H]SR141716A Binding to Cannabinoid CB1 Receptors Is Increased in the Anterior Cingulate Cortex in Schizophrenia.” *Progress in Neuro-Psychopharmacology & Biological Psychiatry* 28 (2): 355–60. https://doi.org/10.1016/j.pnpbp.2003.11.005.

Zhang, Jian-Ping, Juan A Gallego, Delbert G Robinson, Anil K Malhotra, John M Kane, and Christoph U Correll. 2013. “Efficacy and Safety of Individual Second-Generation vs. First-Generation Antipsychotics in First-Episode Psychosis: A Systematic Review and Meta-Analysis.” *The*

International Journal of Neuropsychopharmacology 16 (6): 1205–18.
https://doi.org/10.1017/S1461145712001277.