Investigating Whether Hippocampal, Thalamic and Frontal Dysfunction Underlies Dopamine Dysfunction in First-Episode Schizophrenia Patients

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Abstract

Aim: People with schizophrenia show negative symptoms and impairments in working memory performance but the pathophysiology underlying these remains incompletely understood. There is consistent evidence for altered brain activation in a network including the dorsolateral prefrontal cortex (DLPFC), hippocampus and thalamus during working memory tasks in patients with schizophrenia. However, these studies were largely conducted in patients taking antipsychotic treatment. Thus, it is not clear

1. if there is altered neural function during working memory performance in patients not taking antipsychotics,

2. if antipsychotic treatment changes this,

3. and if it is related to negative symptoms.

Striatal dopamine is involved in regulating information flow from cortical regions involved in working memory performance, and whilst dysfunction is consistently seen in schizophrenia, it is ambiguous if this is associated with altered neural response in other brain regions during working memory performance. To address these issues, I investigated whether working memory load-related neural activity was reduced in drug free first episode patients, and if it was related to negative symptom severity, or striatal dopamine synthesis capacity. Finally, I aimed to determine whether antipsychotic treatments affected neural responses during working memory performance and whether differences in neural responses are linked to clinical change.

Method: I studied antipsychotic-naïve or medication-free patients with first episode psychosis (FEP) (n=45) and healthy control (HC) volunteers (n=33) for baseline and

longitudinal assessments. All three experimental chapters shared the same participants and datasets. Participants were studied using functional magnetic resonance imaging whilst performing the N-Back working memory task to index the blood oxygen level dependent (BOLD) response. Participants also received [^{18}F]DOPA PET imaging to index striatal dopamine synthesis capacity (Ki^{cer}). Negative symptoms were measured using the Positive and Negative Syndrome Scale (PANSS), Negative Syndrome Scale at baseline and at follow up (median = 39 days after baseline).

Results: Of the total FEP (n = 45) and HC (n = 33), the experiments retained the following number of participants after analysis was completed:

- Experiment 1 (see chapter 2) FEP (n = 29) and HC (n = 30),

- Experiment 2 (see chapter 3) FEP (n = 24), HC (n = 18) and

- Experiment 3 (see chapter 4) FEP (n = 16).

Patients displayed attenuated load-related responses (2-Back>1-Back) relative to controls in the thalamus and hippocampus. PANSS negative symptom scores did not correlate with these reduced responses. There were no significant differences in dopamine synthesis capacity between patient and controls, nor were there significant correlations between activation during the N-Back working memory task and Ki^{cer} in either group, or between groups. Treatment with antipsychotic medication was not associated with a significant change in activation from baseline to follow-up in patients after a median of 39 days of treatment. Finally, there were no significant correlations between change in negative symptoms and change in responses during the working memory task.

Discussion: Working memory impairments seen in psychosis may be related to altered hippocampal and thalamic functioning, but these do not seem to be associated with either negative symptoms or striatal dopamine function. There was no evidence that antipsychotic treatment affected neural responses during working tasks, and symptomatic improvement was not associated with altered activation. This suggests that antipsychotic medication does not have significant effects on the neural substrate of working memory in patients with psychosis.

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1. Introduction

1.1. General Introduction

My thesis focuses on psychosis, which is a core feature of schizophrenia and several other mental illnesses. The goal of the research presented here is to focus on patients experiencing their first episode of psychosis in order to clarify some of the pathophysiological features. The reason why I am doing this specifically is that there are fewer confounding factors during the first episode of illness than the chronic phase [1]. As the illness progresses, most patients will be treated continuously with antipsychotic medication, which may have off target effects. The long-term effects of illness can also result in secondary effects [2]. These may interfere with the assessment of the pathology and treatment of psychosis in chronic patients. The best way to decipher these secondary effects from the main disease is to look at patients who have just become ill and therefore not treated yet: first episode treatment-free patients with psychosis.

Outcomes of research studies in chronic patients are not necessarily applicable in first episode patients due to antipsychotic medication. Conducting research in treatment naïve patients experiencing their first episode of psychosis however, gives rise to some practical and ethical issues such as capacity and consent. Despite these complications, I was able to recruit a large sample of patients, with most being able to perform the experiments.

Before discussing the studies and their backgrounds, a brief overview of schizophrenia and psychosis is provided.

1.2. Schizophrenia – epidemiology, symptoms, and diagnosis

1.2.1. Epidemiology

Schizophrenia is the most common psychotic disorder in the world. It is also one of the top ten causes of global disease burden amongst adults and is a leading cause of disability and mortality [3]. It has a lifetime prevalence of 0.7%, mainly affecting adults [4] with the World Health Organization estimating a global prevalence of 21 million people [5]. Cognition is impaired in schizophrenia with function about 0.5-1.5 standard deviations below healthy matched control participants depending on the cognitive task [6]. In men, the onset is generally in the late teens to early twenties, whilst in women it is early to late twenties [7]. Patients with schizophrenia have a life expectancy 12-15 years less than the general population because of a higher suicide rate and physical health problems [8]

1.2.2. Symptoms

In schizophrenia, symptoms are divided into positive, negative, cognitive and mood [9], with cognitive impairment appearing early on in the course of the illness [10]. According to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, (DSM-5) [9], positive symptoms comprise of auditory, delusions, and disorganized speech and behavior, whilst negative symptoms include a reduction in emotional range, loss of interests and drive, poverty of speech, and tremendous inertia.

1.2.3. Cognitive Symptoms

Dysfunction in attention, executive function, working memory, verbal learning, processing speed, and visual learning, with a considerable deficit in planning, abstract thinking, reasoning and problem solving have been widely documented in schizophrenia [11]. Some estimates suggest that up to 98% of patients with schizophrenia have such impairments [12] and these

cognitive deficits are amongst the first signs in people who go on to develop schizophrenia

[13]. Furthermore there is evidence of cognitive impairments in both the prodromal phase

and throughout the course of schizophrenia that more than justifies the inclusion of cognitive

deficits as a core component of this illness [14]. Significant correlations have been shown

between the functional outcome of deficits with independent living, social cognition,

employment, and social functions [15-17]. Cognitive deficits are noticeable, despite patients

being clinically stable and are likely to contribute to disability [18].

1.2.4. Diagnosis

The DSM-5 states that at least two of the following symptoms must be present to meet the

criteria for a diagnosis of schizophrenia: delusions, hallucinations, disorganized speech,

disorganized or catatonic behavior, and negative symptoms [19]. One of the presenting

symptoms must be one of delusions, hallucinations, or disorganized speech. The patient

must have at least one month of active symptoms and the symptoms must persist for six

months.

1.2.5. Etiology

The etiology of schizophrenia is not yet clear and is likely to be multifactorial. Genetics can

play a large part in the risk that an individual may go on to develop schizophrenia [20]. Whilst

the lifetime risk of developing schizophrenia is about 0.7% [21] for the general population,

this figure is increased to roughly 10% in those who have a first degree relative with

schizophrenia [22]. Those with a monozygotic twin have a 50% chance and dizygotic twins

have a slightly higher risk of developing the disease than other siblings at about 12% [23].

22q11.2 deletion syndrome is the largest single, known genetic risk factor for the

development of schizophrenia [24, 25]. Evidence from post-mortem, genetic, and animal

studies have identified several other susceptibility loci for schizophrenia, which include:

- disrupted-in-schizophrenia-1 (DISC1 [26]),

- neuregulin 1 (Nrg1) [27] and its receptor ErbB4 [28],

- dysbindin-1 [29],

- catechol-O-methyl transferase (COMT) [30],

- brain-derived neurotrophic factor (BDNF) [31],

- and Akt [32].

Additionally, environmental factors also play a large part. These include obstetric

complications (such as intrauterine oxygen deprivation [33], and prenatal infection during the

early months of pregnancy [34]), cannabis use [35, 36], difficult childhood [37], stress [38],

and parental socioeconomic status [39]. These factors and related pathways interact closely

with dopaminergic, glutamatergic and gamma-aminobutyric acid (GABA) neurotransmitter

systems [40]. Ultimately, at present, it is not understood exactly how these risk factors

combine and contribute to the development of schizophrenia.

1.3. Pathophysiology- Brain Systems, Cognitive Impairments and Behavioural Consequences

In this section, I will discuss the pathophysiology of schizophrenia. I will start with the

glutamate theory of schizophrenia, before moving onto the GABA-glutamate theory detailing

GABA in the Prefrontal Cortex (PFC). Next, I will provide an overview of dopamine and

discuss the dopamine hypothesis including cortico-striatal loops, imaging, evidence that

striatal dysfunction can disrupt working memory performance and hypofunction of dopamine in the PFC. In turn, I will detail the serotonin and acetylcholine theories of schizophrenia.

I will then discuss alternative proposals regarding the pathophysiology of cognitive impairments in schizophrenia, covering the hypofrontality model (also known as the frontal lobe model), temporal lobe model and how these two main theories of cognitive dysfunction in schizophrenia developed into a model that integrates both prefrontal and temporal-limbic activity. Finally, I will discuss the behavioural consequences of pathophysiological changes in schizophrenia.

1.3.1. The Glutamate Hypothesis of Schizophrenia:

Glutamatergic neurons account for 60-80% of total brain metabolic activity [41]. Glutamate is the primary neurotransmitter in the brain [42] and is used by all pyramidal cortical cells [42] with glutamate receptors divided into ionotropic or metabotropic [42]. Ionotropic receptors consist of a series of ion channels that control the flow of sodium, calcium and potassium ions in and out of the cell [42] and when activated, they have a rapid fast ion flow [42]. Ionotropic glutamate receptors are called after the agonists initially found to selectively activate them [43]: NMDA (N-methyl-D-asparate), AMPA (a-amino-3-hydroxy-5-methyl-4-isoazolepropionic acid) and kainite [44, 45]. Metabotropic receptors are connected to G protein-coupled receptors (GPCR) and neurotransmission is slow necessitating the GPCR to interact with a G-protein to either increase or decrease the level of a second messenger [46]. Metabotropic receptors are further subdivided into group I (post-synaptic) and group II and III (pre-synaptic) [46].

The original glutamate hypothesis proposed that there was a simple deficit in glutamatergic neurotransmission in schizophrenia. One of the earliest findings of glutamate abnormalities was reduced cerebrospinal fluid glutamate levels in patients with schizophrenia[47]. The hypothesis has been refined in more recent years and is now called the NMDA receptor dysfunction hypothesis. It concentrates primarily on NMDA receptor dysfunction [48] with evidence coming from human [49, 50] and animal [51, 52] studies of non-competitive NMDA receptor (NMDAR) antagonists. Ketamine and phencyclidine (PCP) administration produce psychological effects that closely resemble both positive and negative symptoms that occur in schizophrenia, [50, 53, 54]. It is not clear how NMDAR antagonism brings about decreases in GABAergic interneuron activity. It has been hypothesised that they may have preferential effects on NMDA receptors expressed on GABA neurons [55], but this theory has been disputed [56]. An alternative theory is that NMDA receptor antagonist-induced-changes in reactive oxygen species levels might be a main component of this mechanism as reducing superoxide levels prevented ketamine-induced changes in interneuron activity [57]. Additionally, inhibiting reactive oxygen species formation stops the behavioural effect of NMDA receptor antagonists in animals [58-61].

1.3.2. GABA-Glutamate Theory of Schizophrenia

GABA is the main inhibitory neurotransmitter in the brain and is synthesized from glutamate by the enzyme L-glutamic acid decarboxylase. Glutamate not only act as a neurotransmitter, but is involved in nitrogen metabolism, protein synthesis and is a precursor to GABA [62]. A loss of GABA signalling is a prevalent theory for the pathogenesis of schizophrenia. This theory proposes that reduced GABA signalling in frontotemporal brain areas underlies the pathogenesis of schizophrenia [63-65] and supported by post-mortem literature. This demonstrated that patients with schizophrenia (compared to healthy controls) had lower

levels of the GABA membrane transporter (GAT1) [66], lower mRNA and protein levels of the

synthetic enzyme GAD67 [67], and lower cell density and expression of GABAergic

interneurons [65, 68]. In vivo studies have reported a decrease in concentrations of GABA in

cerebrospinal fluid concentrations in first-episode patients with psychosis [69].

As GABA signalling interacts very closely with dopaminergic and glutamatergic systems,

models of GABAergic dysfunction may aide understanding of schizophrenia [70]. Data from

post-mortem studies indicate that schizophrenia is linked with brain GABAergic dysfunction

with differences in GABA receptor subunit expression and reduced GABAergic cell types in the

brains of patients with schizophrenia [70]. During adolescence, it is also known that the GABA

system undergoes changes and that during this time, patients often begin to show symptoms

of psychosis [71]. GABA dysfunction is believed to cause the disinhibition of glutamatergic

pyramidal neurons and a deficit of synchronous cortical activity [65, 72].

1.3.2.1.   Glutamate/GABA Balance on the Disruption of Frontal Cortex Function

Support for hypofunction of glutamate and GABA transmission in the forebrain has been

suggested as one of major contributors to the onset of schizophrenia [73]. Genetic studies

have shown disrupted mutations in neurodevelopmental proteins at glutamatergic and

GABAergic synapses which are theoretically responsible for the synaptic abnormalities seen in

schizophrenia [74, 75]. Magnetic Resonance Spectroscopy (MRS) can measure glutamate and

GABA levels in frontal cortical regions but the MRS literature on glutamate and GABA levels in

schizophrenia is mixed [76, 77]. This variability may be because the peaks of glutamate and

GABA measured in MRS are hard to distinguish at commonly used magnetic field strengths of

1.5 or 3 Tesla (T). Recent 7T MRS studies have been more consistent, reporting lower

glutamate and GABA levels in the frontal cortex of patients with schizophrenia [78, 79]. MRS

measures total tissue levels of glutamate and GABA which includes levels associated with

metabolism and not just neurotransmission.

Positron Emission Tomography (PET) radioligands might overcome these limitations by

directly indexing the availability of GABA in the synapse. For example, studies using this

approach support the idea that GABA synthesis in the frontal cortex is impaired in

schizophrenia subjects [80]. Glutamate dysfunction in the prefrontal cortex may lead to

dopamine dysregulation in the striatum, with NMDA hypofunction making the dopamine

system more responsive to the effects of psychological stress. [81]. Dopamine transmission

in the prefrontal cortex is regulated by GABAergic firing and a GABA interneuron deficit in

schizophrenia has been suggested to underlie some clinical symptoms [71, 82]. Future

studies using other novel PET radioligands may provide more specificity to index the neural

circuitry responsible for working memory in schizophrenia subjects *in vivo*.

GABA-A antagonists disrupt prepulse inhibition when injected into the rodent medial

prefrontal cortex, and through their action on the dopamine system, this can be reversed

with D2-blocking antipsychotic drugs [82]. In an associated theory (from the

methylazoxymethanol (MAM) acetate rodent model of schizophrenia), Lodge and Grace

postulated that increased hippocampal glutamatergic outputs, occurring secondary to

reductions in hippocampal parvalbumin staining GABAergic interneurons in schizophrenia,

lead to increased striatal dopamine activity [83, 84].

1.3.3. Dopamine Overview

1.3.3.1. The Dopamine System

The dopamine system originates in the midbrain and dopaminergic projections from midbrain dopamine cells are divided in nigrostriatal, mesolimbic, mesocortical and tuberoinfundibular.

i. The **nigrostriatal** pathway has projections from the substantia nigra part of the midbrain to the caudate nucleus and putamen (the more dorsal posterior part of the striatum; sensorimotor striatum) that project via the pallidum and thalamus to the motor cortex. Reduced dopamine levels in this pathway are believed to affect the extrapyramidal system, leading to motor symptoms [85].

ii. The **mesolimbic** pathway projects from the ventral tegmental area (VTA) of the midbrain to the ventral striatum including the nucleus accumbens (limbic striatum). The ventral striatum also receives subcortical input from the hippocampus [86]. Both the hippocampus and the prefrontal cortex regulate midbrain dopamine neurons via glutamatergic projections to the midbrain [87-89]. Dopamine dysregulation has been associated with primary or secondary negative symptoms [90].

iii. The **mesocortical** pathway extends from the VTA to the prefrontal cortex where it modulates cognitive function and can enhance the efficiency of working memory [91]. It is believed that reduced mesocortical dopamine levels cause negative and cognitive symptoms [92]

iv. Reduced dopamine levels in the **tuberoinfundibular** pathway, from the hypothalamus to the pituitary gland, are thought to result in elevated prolactin levels, leading to amenorrhea, reduced libido and galactorrhea [93, 94].

1.3.3.2.   The Striatum

The striatum is an essential part of the corticobasal ganglia circuitry and has been divided into three subdivisions based on its anatomical connectivity: limbic (processes emotional information) [95, 96], associative (processes cognitive information) [97] and sensorimotor functional subdivisions [98]. Previously it was believed that these corticostriatal loops worked in parallel, known as the parallel processing model [98, 99]. More recent methodological developments have further refined our understanding of corticostriatal architecture, suggesting that corticostriatal pathways overlap [100, 101]. Cluster analysis of corticostriatal input patterns has additionally shown a fourth subdivision in the most caudal part of the striatum [97].

Motivational behaviour and information processing are facilitated by cortico-striatal-pallidal-thalamo-cortical loops (see Figure 1). These circuits comprise of glutamatergic projections from cortical regions for example, dorsolateral, sensorimotor, and orbitofrontal cortices to the striatum. The striatum sends inhibitory GABAergic projections to the pallidum that it itself projects to the thalamus. The thalamus sends glutamatergic projections back to the cortex.

- The striatum receives projections from the cortex.
- It processes this information and feeds it back to the cortex, via the thalamus.
- Dopamine provides essential modulatory input to these cortico-striatal-pallidal-thalamo-cortical loops.
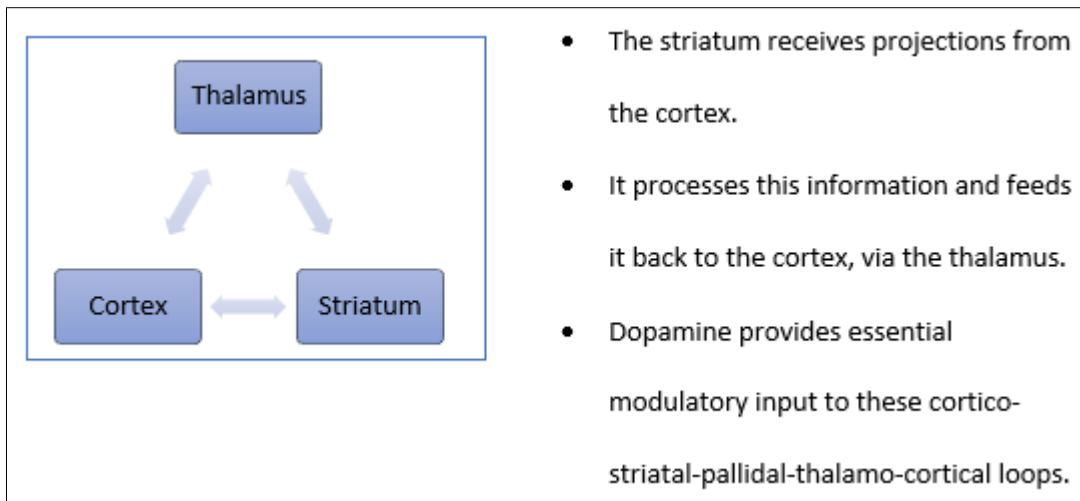
Figure 1: Cortico-striatal-pallidal-thalamo-cortical loop

1.3.3.3. Corticostriatal Loops

There are three well-known corticostriatal loops: the associative, motor, and limbic loops. Figure 2 provides a diagrammatic overview. Three distinct cortical-to-subcortical loops are shown by the colour coding. There are common pathways within the basal ganglia, connecting the striatum (bottom left), the external and internal globus pallidus, and the subthalamic nucleus (bottom right Figure 2). The basal ganglia project to the thalamus (the middle of Figure 2) and has multiple efferents and afferents to the three brain systems. The loops overlap with important sensorimotor connections of the cerebral cortex, representing an important, parallel organization of the brain. The loops are anatomically and functionally segregated, and relevant for specific domains of complicated human communication [102-104]. The limbic loop includes, but is not limited to, the hippocampus and amygdala (dark blue, left panel of Figure 2), which have efferents and afferents to the orbitofrontal cortex (light blue in Figure 2). The limbic loop also includes the ventral striatum.
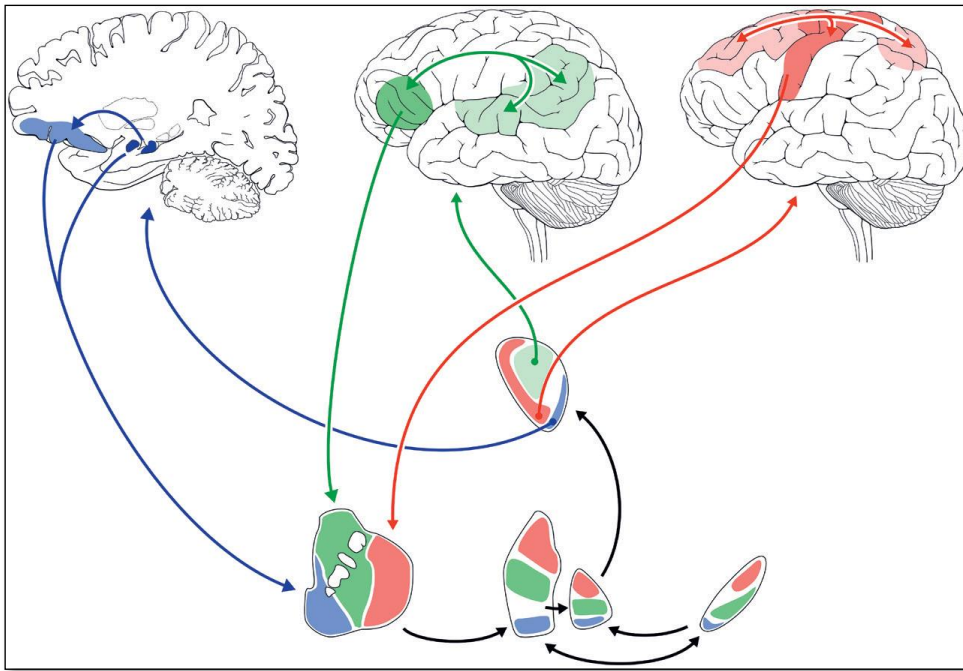
Figure 2 (taken from [105]): Corticostriatal loops and their respective overlap with corticocortical sensorimotor circuits. **The blue loop: limbic loop; the green loop: associative loop and its links with the language system; the red loop: motor loop and the cortical executive system. The coloured arrows indicate distinct connections, whilst the black arrows suggest common connections. Please note that the basal ganglia (on the bottom row) and thalamus (in the middle row) has distinct projections for these the three systems. Thalamocortical connections are distinct, numerous, and reciprocal.**

1.3.3.4.   The Dopamine Hypothesis of Schizophrenia

For the past fifty years, dopamine (or more precisely dopaminergic hyperactivity at D2 receptors in the mesolimbic pathways) has been linked to psychosis, and particularly positive symptoms [106, 107]. Drugs that block D2 receptors in the ventral striatum (part of the mesolimbic dopamine pathway) have been the foundation of treatment of broadly all forms of psychosis [106, 107]. More recently, this has been found to be inaccurate as it appears that dopamine abnormalities in schizophrenia are more marked in the dorsal striatum (which

predominantly receives dopamine projections from the substantia nigra) [108, 109]. In contrast, the mesolimbic pathway to the ventral striatum appears largely unaffected in schizophrenia [109]. Hyperactivity of dopamine in the dorsal striatum from neuronal projections from the midbrain is thought to be responsible for psychotic symptoms such as hallucinations and delusions whilst also contributing to negative and cognitive symptoms in schizophrenia [110] (see Figure 3).
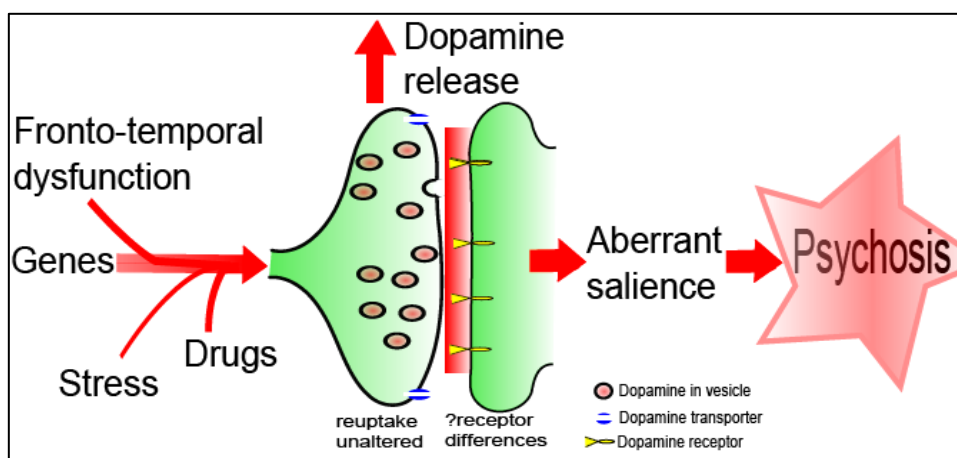
Figure 3: From Dopamine Dysregulation to Psychosis. **The dopamine hypothesis of schizophrenia proposes that dopamine dysregulation results from many factors interacting and leading to a change in the salience of stimuli thereby resulting in psychosis. Disruption of dopamine signalling is also postulated to contribute to cognitive and negative symptoms. Antipsychotic medication acts downstream of the primary dopaminergic dysregulation (from Howes (2009) [111]).**

The dopamine hypothesis of schizophrenia developed from three main areas of evidence: amphetamine models, antipsychotic actions, and clinical imaging findings. The administration of amphetamines increases extracellular concentrations of dopamine which can produce auditory hallucinations and paranoid psychosis in healthy people comparable to psychosis

[106, 107, 112] (see [107, 113] for review).   Amphetamines can also worsen psychotic symptoms in patients with schizophrenia [114, 115].

The discovery that antipsychotics affect the dopamine system, with their clinical potency being directly related to their affinity for dopamine receptors [116-119], provided further evidence for the dopamine hypothesis. More evidence comes from studies of drugs that reduce dopamine levels, for example, alpha-methyl-para-tyrosine and reserpine [120], which were shown to reduce psychotic symptoms [121-124]. When it was initially developed, the dopamine hypothesis postulated that schizophrenia arose from anomalies in dopamine receptor density [125, 126] and that psychosis was due to overactivity in the mesolimbic dopamine pathway [127]. Current neurochemical imaging results however, suggest that it is within dorsal regions of the striatum that dopaminergic abnormalities are highest [108]).

Post-mortem studies offered the first direct evidence of the anatomical localisation of the dopaminergic system in the brain and of its dysfunction. These studies demonstrated raised levels of dopamine in the striatum of medicated patients with schizophrenia [128].   Levels of the rate-limiting enzyme, tyrosine hydroxylase, were found to be raised throughout the striatum [129], as was dopamine receptor density [130]. However, the post-mortem studies are limited as the majority of patients were treated with antipsychotic medication, which might have upregulated dopamine receptors [131] or altered presynaptic dopamine function [132].

1.3.3.5.   Physiological Alterations in Striatal Dopamine in Schizophrenia

Meta-analysis has found overall that that there is a large effect size in elevation in dopamine synthesis and release capacity in schizophrenia relative to controls [133] (see Figure 4).

Currently, there is no evidence of major differences in dopamine D2/3 receptors in people with schizophrenia relative to controls (see Figure 5), although it is possible that increased receptor occupancy by elevated endogenous dopamine levels might mask this [134] or that the group differences in the affinity state of the receptor are not detected with the antagonist ligands commonly used [52, 133, 135]. Striatal dopamine synthesis capacity has also been found to be elevated in people at ultra-high risk (UHR) of developing psychosis [136-142], although differences have not been seen in UHR subjects who do not transition to psychosis [143], suggesting dopamine dysfunction may be specific to transition to psychosis [144]. The elevation in striatal dopamine synthesis capacity has been reported in first episode patients [145-148] as well as in chronic patients [149, 150] compared to healthy matched controls. There is also a large body of evidence from *in vivo* positron emission tomography studies which demonstrates that there is an increase in stimulant-induced striatal dopamine release in schizophrenia. [133, 151, 152]. Together these lines of evidence indicate dysregulation of presynaptic production and release of dopamine in the striata of people with schizophrenia.

Study	Year	Std effect	95%–C.I.	Z–Value	p–Value
Reith	1994	1.5194	0.3748 – 2.6640	2.6018	0.0093
Hietala	1995	0.9004	–0.1639 – 1.9647	1.6581	0.0973
Dao–Castellana	1997	0.3508	–0.7480 – 1.4495	0.6257	0.5315
Breier	1997	0.8766	0.0201 – 1.7330	2.0060	0.0449
Lindstroem	1999	1.0054	0.1152 – 1.8957	2.2136	0.0269
Hietala	1999	1.0246	0.1486 – 1.9005	2.2924	0.0219
Laruelle	1999	0.9132	0.4207 – 1.4058	3.6342	0.0003
Elkashef	2000	–0.1313	–0.8375 – 0.5749	–0.3644	0.7156
Abi–Dargham	2000	1.0847	0.3849 – 1.7844	3.0382	0.0024
Meyer–Lindenberg	2002	1.8245	0.4779 – 3.1711	2.6556	0.0079
McGowan	2004	1.5549	0.7028 – 2.4070	3.5766	0.0003
Kumakura	2007	0.0990	–0.7595 – 0.9576	0.2261	0.8211
Nozaki	2008	0.1329	–0.5046 – 0.7704	0.4087	0.6828
Howes	2009	1.1849	0.2484 – 2.1215	2.4797	0.0131
Kegeles	2010	0.6136	–0.0549 – 1.2821	1.7989	0.0720
Summary		0.7919	0.5173 – 1.0666	5.6518	<0.0001

Figure 4 - Studies of presynaptic dopaminergic function: Forrest plot showing the effect size and 95% confidence intervals of the difference between patients with schizophrenia and controls by study. There was evidence of a significant elevation in schizophrenia with a summary effect size of *d*=0.79. (Taken from Howes et al. (2012) [133]).

(Intentional break)

Study	Year	Std effect	95%–C.I.	Z–Value	p–Value
Crawley	1986	0.8889	0.0665 – 1.7113	2.1185	0.0341
Farde	1990	0.3447	–0.2968 – 0.9862	1.0532	0.2922
Martinot	1990	0.1116	–0.6892 – 0.9124	0.2732	0.7847
Martinot	1991	0.3969	–0.3000 – 1.0939	1.1163	0.2643
Tune	1993	1.6024	0.8974 – 2.3075	4.4548	<0.0001
Hietala	1994	0.3337	–0.4963 – 1.1637	0.7879	0.4307
Martinot	1994	0.0000	–0.8765 – 0.8765	0.0000	1.0000
Pilowsky	1994	–0.1042	–0.7244 – 0.5161	–0.3292	0.7420
Pedro	1994	0.0927	–0.6667 – 0.8522	0.2393	0.8108
Nordstrom	1995	0.7003	–0.3790 – 1.7796	1.2717	0.2035
Laruelle	1996	0.5116	–0.2412 – 1.2644	1.3319	0.1829
Okubo	1997	0.2188	–0.4460 – 0.8836	0.6450	0.5189
Breier	1997	0.0000	–0.8181 – 0.8181	0.0000	1.0000
Abi–Dargham	1998	0.0457	–0.6701 – 0.7614	0.1251	0.9005
Abi–Dargham	2000	0.3154	–0.3420 – 0.9727	0.9402	0.3471
Yang	2004	–0.1397	–0.9588 – 0.6795	–0.3342	0.7383
Talvik	2006	–0.2016	–0.8661 – 0.4630	–0.5945	0.5522
Corripio	2006	2.3291	1.3689 – 3.2892	4.7543	<0.0001
Schmitt	2009	–0.9150	–1.6895 – –0.1404	–2.3153	0.0206
Kessler	2009	–0.4429	–1.2888 – 0.4030	–1.0261	0.3048
Kegeles	2010	0.0735	–0.5800 – 0.7271	0.2205	0.8255
Kegeles	2010	0.0155	–0.5825 – 0.6134	0.0507	0.9595
Summary		**0.2587**	**0.0010 – 0.5165**	**1.9674**	**0.0491**

Figure 5: Studies of D2/3 receptor availability: Forrest plot showing the effect size and 95% confidence intervals of the effect sizes by study. There was evidence of a small increase in D2 receptor availability in schizophrenia with a summary effect size (lozenge) of d=0.26 (Taken from Howes et al. (2012) [133]).

1.3.3.6. Dopamine Imaging Findings in Schizophrenia and the Link to Negative and Cognitive Symptoms

In this section, I will discuss the evidence supporting dopamine having a role in negative and cognitive symptoms in schizophrenia.

Hyperdopaminergia in the striatum might plausibly result in cognitive impairments either by potentially driving cortical dopamine dysregulation or by interrupting signalling between the

frontal cortex and associative striatum [153]. Animal models of D2 receptor overexpression in

the striatum have been designed to mimic elevated striatal dopamine signalling seen in

schizophrenia [153, 154]. Using this model, studies found that striatal D2 overexpression

reduced in cortical dopamine turnover and cognitive deficits [154]. These abnormalities

continued even after the normalisation of striatal dopamine transmission, indicating that

whilst elevated striatal dopamine signalling leads to cognitive impairments, consequent

adaptive changes might be the cause of their persistence [154]. The possible role of the

striatum has gained evidence from *in vivo* studies demonstrating that decreased connectivity

between the striatum and substantia nigra is linked to greater severity of cognitive deficits in

schizophrenia [155, 156].

There is also evidence that striatal dopamine dysfunction might directly contribute to

negative symptoms. Using probabilistic learning tasks, studies have shown that negative

symptoms in people with schizophrenia might be related to impaired reward-based learning

[157-160]. Numerous studies have shown that the striatum plays a main role in the

organisation of this type of behaviour [161], and it has been hypothesised that excessive

dopamine release may disrupt adaptive striatal dopamine release, thus contributing to these

behavioural impairments in schizophrenia [160]. This is consistent with neuroimaging papers

demonstrating decreased striatal activation during reward processing in patients [162, 163].

1.3.3.7.   Working Memory and Dopamine

As striatal dopamine activity can modulate working memory performance [228], there have

been suggestions that prefrontal activation dysfunction in schizophrenia might be related to

striatal dopamine dysfunction [229]. The pharmacological manipulation of dopamine activity

in studies with non-human animals [164] and healthy human subjects [165] demonstrates

changes in prefrontal function during a working memory task. As pharmacological

manipulations are not regionally selective, this could be due to altered dopamine

transmission throughout the brain. However, regionally selective manipulations of dopamine

transmission in mice enable the role of dopamine neurotransmission in the striatum to be

specifically investigated. Mice with selective overexpression of D_2 receptors in the striatum

have shown reduced performance on working memory tasks, indicating that increased striatal

dopamine neurotransmission can impair working memory [154].

Cortical projections to the striatum in tracing studies show a topographical distribution across

the striatum [103, 166, 167]. Projections from limbic regions, such as the hippocampus,

project to the anterior and ventral regions of the striatum [83]. The hippocampal

hyperactivity seen in rodent models and in patients with schizophrenia is thought to

contribute to dopamine hyperfunction [83] leading to cognitive impairment. Glutamatergic

neurotransmission in the hippocampus might be related to abnormal dopaminergic

neurotransmission in schizophrenia and psychosis [72]. These results are further supported

by the presence of extensive projections from glutamatergic output neurons in the

hippocampus to the striatum in the human brain [168].

Elevated dopamine synthesis and release capacity has been found in the associative striatum

in patients with psychosis [52]. This striatal region receives projections from the dorsolateral

prefrontal cortex [136, 137, 169-171] to the caudate and anterior putamen, suggesting a link

between dorsolateral prefrontal cortex and striatum in schizophrenia. It has been theorised

that prefrontal cortex dysfunction can lead to striatal dopamine dysfunction [172] and

elevated striatal dopamine transmission in schizophrenia might be the consequence of

dorsolateral prefrontal cortex abnormalities [173]. It has been further suggested that

dopamine hypofunction in the dorsolateral prefrontal cortex (DLPFC), can account for cognitive symptoms [174].

1.3.3.8. Dopamine Hypofunction in the Dorsolateral Prefrontal Cortex (DLPFC)

It has been argued that mesocortical dopamine hypofunction in the dorsolateral prefrontal cortex (DLPFC) might underlie cognitive symptoms in schizophrenia [175]. This idea emerged from evidence demonstrating that working memory is impaired in schizophrenia [176], and that working memory critically depends on optimal dopamine transmission in the prefrontal cortex in non-human primates [177-184]. Additional evidence comes from studies showing working memory can be improved with dopamine agonists [185-188]. There is also evidence from PET studies in patients with schizophrenia that have investigated changes in D1 prefrontal receptor availability [189]. Abi-Dargham et al (2002) [189] reported a significant elevation in D1 receptor availability in patients in the DLPFC of compared with control participants. The authors suggest that increased D1 receptor availability seen in patients with schizophrenia might be a compensatory (but ineffective) upregulation secondary to sustained deficit in mesocortical dopamine function.

Abi-Dargham et al (2012) [190] reported a significant increase in prefrontal D1 binding potential in patients with schizophrenia compared to controls and only in drug-naive but not drug-free patients. They surmised that upregulation in frontal D1 receptors availability in drug-naive patients might reflect low frontal dopamine in schizophrenia and could be corrected and normalized with long-term antipsychotic treatment. In contrast, Okubo et al (1997) [191] report a significant decrease in D1 receptor levels in schizophrenia and suggest that dysfunction of D1 receptor signalling in the PFC might contribute to the negative

symptoms and cognitive deficits seen in schizophrenia. As the patient group in the study by

Okubo et al (1997) had received antipsychotic treatment, this difference from the findings in

antipsychotic naïve patients reported by Abi-Dargham et al (2012) could be accounted for by

antipsychotic effects, as suggested by Abi-Dargham et al (2012), although it has yet to be

shown in longitudinal studies in patients.

A key study was conducted by Slifstein et al (2015) [192], who examined for the first time the

in vivo capacity for dopamine release in the prefrontal cortex in patients with schizophrenia

using an amphetamine challenge [192]. They used PET and MRI separately to look at the

relationship between cortical dopamine release and DLPFC activity using the [^{11}C]FLB457

tracer and two PET scans, one before, and one 3 hours after the administration of an

amphetamine challenge in patients with schizophrenia and in healthy control participants. In

addition to this, they used the self-ordered working memory task (SOWT) during separate

functional MRI scans also conducted before and after amphetamine administration. In terms

of dopamine receptor availability in patients relative to controls, Slifstein et al. (2015) [192]

found that that baseline DLPFC BP_{ND} did not differ significantly between groups. However, the

change in binding potential (ΔBP_{ND}) was significantly lower in schizophrenia, indicating

blunted dopamine release in patients in the DLPFC. This supports the cortical dopamine

hypofunction hypothesis in schizophrenia.

In addition, Slifstein et al (2015) found that cortical dopamine release (measured using

[^{11}C]FLB 457) was associated with BOLD activity changes during a cognitive task (SOWT) in

both patients and healthy controls. However, there were no significant correlations between

working memory performance and DLPFC binding potential (BP_{ND}), percent change in binding

potential (ΔBP_{ND}), distribution volume (V_T), or percentage change in distribution volume (Δ

V_T) in patients with schizophrenia. This could be due to a lack of statistical power given the relatively modest sample size (patients = n=20 ((with schizophrenia (n=19) or schizoaffective (n = 1)), healthy controls n = 21), or could imply that other factors contribute to behavioural performance. One possibility is that other brain regions can compensate for impairments in frontal cortical function to maintain behavioural performance.

Slifstein et al (2015) postulated that the relationship between dopamine release and BOLD activity implies that variations in dopamine release in the DLPFC might regulate the strength of the haemodynamic response to cognitive processing challenges placed on the pathways in the DLPFC. Their study did not use a combined PET/MRI scanner to simultaneously test if dopamine release is reduced in the DLPFC during a working memory task, so it remains unknown if frontal dopamine release during working memory performance is blunted in schizophrenia. Future work is needed to investigate this.

Overall, the findings to date showing frontal cortical hypodopaminergia associated with the neural responses seen during working memory are consistent with the idea that frontal hypodopaminergia contributes to cognitive impairments in schizophrenia. To date though there is only the Slifstein et al (2015) study that has measured frontal dopamine release in schizophrenia with an amphetamine challenge, indicating replication is warranted. Moreover, this study found no relationship between frontal cortical dopamine release and working memory performance, which could indicate that other brain regions are involved in determining performance, highlighting the importance of investigating the role of dopamine function in other regions, such as the striatum, and cognitive function in schizophrenia.

1.3.4. Serotonin Theory of Schizophrenia

There is some evidence that the serotonin (5-HT) system might also be involved in formation of psychotic symptoms. Evidence comes from the study of hallucinogens that are 5-HTA-receptopr agonist, such as psilocybin and LSD [193]. These drugs can induce psychopathologies which include visual hallucinations that are akin to the symptoms sometimes seen in the first episode of psychosis [194, 195]. Additionally, these drugs disrupt prepulse inhibition (PPI) through directly stimulating the 5-HT2A receptors [196] [197], leading to a merging of brain networks engaged during active task performance and rest [198]. They also lead to downstream increases in glutamate release [199, 200], which could contribute to glutamate changes seen in patients with psychosis. Serotonergic impairment is believed to contribute to the pathophysiology of schizophrenia, but evidence to support this is mainly based on drug challenges which are indirect indicators of brain serotonin function. In vivo imaging and post-mortem studies have directly indexed serotonin function in patients to address this issue. Selvaraj et al (2014) [201] conducted a systematic review and meta-analysis of post-mortem and molecular imaging studies of serotonin function in schizophrenia. Their meta-analysis revealed an increase, with a moderate to large effect size, in prefrontal 5-HT1A receptors and a decrease in prefrontal 5-HT2A receptors with a large effect size in schizophrenia compared to healthy controls.

1.3.5. Acetylcholine Alterations on PFC Dysfunction

I will now consider the effect of acetylcholine (ACh) alterations on the disruption of frontal cortex function. The prevalent hypotheses of the pathophysiology underlying schizophrenia have mainly focused on monoamines like dopamine and serotonin. There is increasing evidence from clinical and preclinical studies that aberrant acetylcholine signalling might also contribute to the illness [202-204]. The actions of ACh are mediated by two main classes of

receptors: ionotropic nicotinic receptors (nAChRs) and metabotropic muscarinic receptors (mAChRs) (reviewed in [205, 206]). Epidemiological studies have long provided evidence that patients with schizophrenia show greater rates of tobacco smoking than in the general population and have suggested that patients might use nicotine (an agonist of nAChRs) for self-medication [207, 208]. Aberrant signalling through mAChRs has also implicated in schizophrenia. Post-mortem studies of patients have revealed a reduction in expression of these receptors throughout the brain, including the prefrontal cortex and hippocampus [209-211]. Despite these clinical observations, mechanistic links between the pathophysiology of schizophrenia and the cellular actions of ACh have been hard to establish. Recent research has focused mainly on the influence of cholinergic activity on normal behaviours that are known to be disrupted in patients with schizophrenia, such as attention and working memory [212].

Cholinergic activity in the rat neocortex has been linked with control of circuits underlying attention, short-term memory and cue detection, cognitive abilities that are disrupted in patients [213]. Tests of sustained attention are impaired by lesions of cholinergic inputs arising from the basal forebrain [214-216]. Additionally, stimulation of α4β2 nAChRs in the medial prefrontal cortex improves performance in a visual attention task [217], while genetic deletion of these receptors in the mPFC impairs visual attention [218] and auditory discrimination [219]. Taken together, these results imply that cholinergic actions across both metabotropic and ionotropic receptors, and diverse brain areas contribute to cognitive processing.

1.3.6.   Pathophysiology of Cognitive Impairments in Schizophrenia

In this section, I will discuss consider two influential pathophysiological models for cognitive impairments in schizophrenia. Firstly, I will look at the hypofrontality model (also known as the frontal lobe model). Secondly, I will discuss the temporal lobe model. Finally, I will discuss how these theories of cognitive dysfunction in schizophrenia developed into a model that integrates them.

Hypofrontality is defined as a "failure to activate frontal systems during prefrontal-cortex-related cognitive activity" [220]. In schizophrenia this was first observed in resting cerebral blood flow (CBF) studies using the "[113]Xenon" clearance method. Ingvar and Franzen (1974) [221] found that in older patients (mean age 61 years), compared to younger patients (mean age 25 years) there was a decreased gradient of frontal to posterior blood level in brain. This led to the conclusion that schizophrenia was characterised by hypofrontality. There were several resting CBF studies that followed on from this and reproduced this pattern of a diminished gradient of frontal to posterior blood flow [222-226]. A limitation of these studies was a lack of behavioural control over participants mental activity because researchers could not be sure what the specific changes in CBF were related to.

Cognitive activation tasks were used in subsequent studies to address this issue, where changes in CBF were measured in relation to task demand. The Wisconsin Card Sorting Task (WCST) was the most popular of these tasks. In a collection of studies, Weinberger, and colleagues, [222, 227], measured CBF change between the WCST and a control (psychomotor) task in patients and controls. The studies showed that patients did not demonstrate a normal increase in blood flow in the DLPFC during WCST performance. A limitation of these studies was that they averaged activity across multiple trials within a block and were unable to

control for performance at low levels on the WCST. Notwithstanding these limitations, the findings suggest blunted DLPFC responses, consistent with hypofrontality.

Another theory of cognitive impairment in schizophrenia pathophysiology, first proposed during the 1980s, was that it was due to temporal lobe dysfunction [228]. Initial evidence for this came from research into long-term memory (LTM) in schizophrenia using PET and "[113]Xenon" found evidence of decreased CBF and abnormal asymmetries in temporal cortex during memory retrieval tasks [229]. fMRI studies added to these findings to provide evidence of hippocampal and temporal lobe abnormalities. For example, Heckers et al (1998) [230] found patients had reduced hippocampus activation measured using fMRI during a work retrieval task relative to controls.

1.3.6.1.   Hybrid model

Friston et al. (1992) were amongst the first to suggest that frontal and temporal lobe deficits might be linked to an underlying fronto-temporal network dysfunction in schizophrenia [231]. Evidence from people with long term memory impairment showed that hippocampal dysfunction often presents together with disrupted prefrontal function [232]. One study provided evidence that there was an interconnectivity between prefrontal regions and the hippocampus [233]. Evidence that impairment on frontal lobe tasks (e.g., WCST) could result from temporal lobe pathology [234] gave further support for a hybrid model that schizophrenia might be best thought of as a disruption of widely distributed neural networks involving frontal and temporal regions rather than as a disorder of an individual brain region [235]. Further support for this model came from imaging studies using verbal fluency tasks. In healthy volunteers, speeded word production decreased superior temporal lobe activity and increased prefrontal activity [236]. Similar reciprocal relationships between temporal

and frontal activity have been reported in fMRI studies of verbal memory as well [230, 232, 237-239]. Studies of cortical thickness and near-infrared spectroscopy have shown altered fronto-temporal relationships during cognitive tasks in schizophrenia [240, 241] and in those at high risk of developing psychosis [242]. PET studies have also measured fronto-temporal correlations in patients with schizophrenia. Friston et al (1996) [243] found that the negative correlation between prefrontal and temporal activity previously seen in healthy controls was reversed in patients with schizophrenia and the left superior temporal gyrus appeared to be dissociated from the prefrontal systems linked in word generation. One question was whether findings are state or trait related. Wolf et al. (2007) tested this and found evidence of disrupted fronto-temporal connectivity when patients were clinically stable compared to healthy controls, which gives support to the notion that this disruption might be a trait-like variable [244]. The specific molecular abnormalities that underlie altered fronto-temporal function and the way that these may lead to alterations across a range of higher cognitive functions have yet to be elucidated, although cortical blunted dopamine signalling is a candidate (as discussed previously).

This section has sought to provide a review of how two prominent theories of pathophysiology of cognitive impairment in schizophrenia evolved and developed into a hybrid model: the fronto-temporal dysconnectivity model. The hybrid model best accounts for the findings in both frontal and temporal cortices in people with schizophrenia. However, the molecular basis of altered activity in cortical regions is not made clear in these theories and remains an area that needs further investigation.

1.3.7. Behavioural Consequences of Pathophysiological Changes in Schizophrenia

Punishment and reward are vital drivers of behaviour [245]. Reinforcement models of learning validate the relationship between reward, states, and behaviour. Prediction errors allow the value of actions and states to be learnt. They are a main signal in numerous reinforcement learning models. Striatal dopamine is involved in regulating information flow from cortical regions involved in working memory performance. Provided the pivotal role of dopamine in both coding prediction errors and in the cortical depiction of environmental states, many studies have used this framework to look at the behavioural consequences of disrupted dopamine signalling [246].

Cortical D1 receptors play a key role in moulding the accurate neural representation of environmental states, by allowing exact inhibition of neural activity [247]. A reduction in cortical dopamine signalling means that stimuli linked with reward cannot be accurately encoded, essentially foreclosing their ability to guide behaviour [247]. Moreover, a reduction in cortical dopamine signalling might mean that reward-related representations are brief, with the consequence that, even if properly represented, the motivational properties of reward-associated stimuli have a shorter impact [247].

Dopamine neurons fire in reaction to stimuli that have been formerly linked with reward, and steer behaviour towards actions linked with a past reward [248]. A hyperdopaminergic state in the striatum might mean that reward-associated stimuli have decreased motivational influence, as aberrantly high background amounts of dopamine signalling decrease the signal-to-noise ratio of adaptive phasic signalling [160]. This system also has the potential to decrease the appetitive properties of a given reward, thus reducing its impact to mould

future behaviour, and account for negative symptoms such as anhedonia and amotivation [157, 249, 250]. This decreased signal-to-noise ratio might account for the decreased striatal activation to reward observed with fMRI in people with schizophrenia [162].

1.4. Antipsychotic Medications

1.4.1. How they work

Pharmacological treatments have been used to treat psychosis since chlorpromazine was first discovered in the 1950s. Antipsychotic medications have been shown to improve positive symptoms [251], but are either not effective for negative or cognitive symptoms [252] or have modest effects and are difficult to separate from other drug effects [253, 254]. Unlike positive symptoms, negative and cognitive symptoms remain quite stable over time [255] and both are good predictors of functional outcome [256]. Understanding the neural mechanisms underlying cognitive and negative symptoms is therefore important to help identify new treatment approaches.

Pharmacokinetics describes the effect of the body on the drug, and pharmacodynamics describes the effect of the drug on the body. Pharmacokinetics has four phases: absorption, distribution, metabolism and elimination [257]. With pharmacodynamics, there are some factors that influence the effect of the drug on the body: affinity of the drug for the target and secondly, the relative binding affinity for other targets, which influences side-effects. The former describes how well the drug binds to the site of action such as a receptor and the latter describes the drug's affinity for a secondary receptor divided by the affinity for the primary receptor. The primary receptor for antipsychotics is the D_2 receptor [258], where they have an overall blocking or antagonising effect [81]. The other factor that affects pharmacodynamics relates to what the compound does at the receptor or the intrinsic

activity. The compound can act as: a full agonist that stimulates the receptor; an antagonist that reduces the transmission by blocking binding of the endogenous neurotransmitter; a partial agonist that stimulates the receptor but less than the endogenous neurotransmitter and blocks the binding of the endogenous neurotransmitter; or an inverse agonist which reduces the transmission below the neutral point. Antipsychotics are either full antagonists or partial agonists at the D2 receptor.

1.4.2. Mechanisms of Action:

Antipsychotics have varying dopamine, serotonin, norepinephrine, histamine, and acetylcholine receptor affinities [259, 260]. However, a common feature of antipsychotic medication is functional dopamine D_2 antagonism [261]. Differences in receptor binding affinity and relative binding affinity are important factors of an antipsychotics predicted efficacy and side effects [262]. Effective D_2 blockage is achieved at different dose levels and can occur before, around or after the antipsychotic concentration is strong enough to block other receptor systems [262].

The relative binding affinity of antipsychotic medication divides treatment into three main groups: First generation antipsychotics such as haloperidol and perphenazine, which have a high affinity for dopamine receptors (D_2 in particular), which is believed to be associated with a reduction in positive symptoms. Second generation antipsychotic or atypical, medication, such as risperidone and ziprasidone, are both 5-Hydroxytryptamine, 5-HT_{2A} and D_2 antagonists. These treatments bind more tightly to 5-HT_{2A} than to the D_2 receptors allowing for antipsychotic efficacy at lower D_2 blockade [263], resulting in having a lower risk of causing tardive dyskinesia or extrapyramidal symptoms. The third group of antipsychotics include aripiprazole and are D2 partial agonists [261].

1.4.3.   Effect of Antipsychotics on Dopamine Pathways

D_2 receptor antagonism is critical for antipsychotic effect [264, 265]. The dysfunction of each of the midbrain dopamine pathways is linked with specific effects [52, 266, 267]. Elevated dopaminergic transmission in the striatum is related to positive symptoms [268]. Antipsychotic-mediated blockade of this pathway can aid treatment of hallucinations and delusions [269]. Blockade of or dysregulation in the mesolimbic pathway from the ventral tegmental area (VTA) to ventral striatum (including limbic striatum) has been associated with primary and secondary negative symptoms [270]. Effective antipsychotics blocking the mesolimbic pathway successfully manages positive symptoms but may surprisingly worsen negative and cognitive symptoms due to excessive blocking in the mesocortical [271] pathway. However, recent neurochemical imaging findings suggest that dopaminergic aberrations in psychosis are greatest in the nigrostriatal (dorsal region of the striatum), not the mesolimbic pathways as previously thought [108].

1.4.4.   Neuroimaging and Treatment

The heterogeneity of different antipsychotic treatments with different binding affinities and receptor profiles shadows the effect of medications in fMRI investigations. A recent review of fMRI studies of medicated patients with schizophrenia only included studies that had at least two groups of patients on different treatments. It concluded that there was no general effect of medication on BOLD signal but as antipsychotics are potent D_2 receptor antagonists, they may reduce the BOLD signal [272]. fMRI measures changes in oxygenated blood flow with BOLD contract, a correlate of neuronal activity. The combination of neural and vascular events challenges the biological interpretation of BOLD signal.

1.4.5. Antipsychotics and BOLD signal

BOLD signal changes seen in treated or untreated patients with schizophrenia are usually interpreted as alterations in neuronal activity [273]. Changes in the quality or quantity of neurovascular coupling or cerebral vasculature can complicate this simple conclusion. Antipsychotic medication can affect BOLD signal by directly modulating astrocytes, neurons and cerebral blood flow as well as further compound the intricacy of the biological understanding of the BOLD signal [274, 275]. fMRI studies have looked at both the acute effects of antipsychotics in healthy volunteers and at the chronic effects in patients. The acute effects have shown differences in the BOLD signal and tasks show a reduced negative modulation or diminished activation with D_2 antagonism [276-282]. Dopaminergic antagonism appears to alter functional connectivity or temporal coherence in healthy control volunteers [283, 284]. Acute administration of antipsychotics in healthy controls shows that D_2 antagonism decreases BOLD signal, which suggests that changes might be attributable to neural factors [273].

Chronic administration of antipsychotics affects dopamine and is related to signalling in different ways from acute administration. Firstly, chronic administration leads to decreased firing (depolarization inactivation) in the striatum, compared to increase firing of nucleus accumbens and striatal dopaminergic neurons with acute administration [285]. Secondly, chronic administration of fluphenazine or haloperidol might upregulate D_2 receptor density [286, 287]. Thirdly, chronic D_2 antagonism might protect against NMDA-mediated neurotoxicity, a possible mechanism of antipsychotic effect [288].

Neuroimaging studies in schizophrenia have investigated whether antipsychotic medication is able to alter brain activity [272, 289, 290], but there have been varied results. There was no

difference swapping from conventional psychotics to olanzapine [291]. Some researchers

have extended this research to investigate the link between the change in brain function

during a working memory or executive function task and a change in negative symptoms.

Some have shown a normalisation [292-296] whilst others have not [296, 297]. A change in

frontotemporal activation levels with antipsychotic treatment has been reported by several

studies in first episode patients [293, 294, 296, 298]. Enhanced bilateral frontotemporal

function after treatment was reported to be associated with improvement in negative

symptoms and accuracy on the verbal working memory task [298]. In medication-naïve first

episode patients, dorsolateral prefrontal cortex dysfunction was related to the severity of

negative symptoms and disorganisation [299]. The follow-up of this study [292] used a

modified Sternberg working memory task. Patients with no symptomatic improvement ("non-

responders") had abnormal left DLPFC function compared to "responders" and healthy

controls [292]. Negative symptoms in schizophrenia can influence working memory

performance but may be associated with unwelcome antipsychotic effects [300, 301]. The

neural mechanisms underlying cognitive impairments and negative symptoms remain unclear

[81, 136].

1.5. Imaging Techniques

1.5.1. Positron Emission Tomography

The first Positron Emission Tomography (PET) camera was built for human studies in 1973

[302, 303]. It is a method that measures physiological function by examining metabolism,

blood flow and neurotransmitters, using radiolabelled tracers. A tracer or ligand is a biological

compound of interest labelled with a positron-emitting isotope, such as ^{11}C, ^{18}F, ^{15}O or ^{13}N.

These isotopes are used because they have relatively short half-lives (minutes to less than

two hours), allowing the tracers to reach equilibrium in the body, but without exposing the

subjects to prolonged periods of radiation [304]. PET offers quantitative analyses, allowing

relative changes over time to be monitored as a disease process evolves or in response to a

specific stimulus. The PET scan will detect the radioactivity emitted after a small amount of a

radioactive tracer is injected intravenously into a peripheral vein.

The process of the PET scan is detailed below in Figure 6 which shows that, first, the

radioisotope linked to a ligand emits a positron. Secondly, this positron collides with an

electron and this process converts mass to energy in the form of two photons. Thirdly, the

PET camera uses scintillation crystals positioned around the participant to detect the

photons. Finally, the crystals absorb the photons, generating light that is changed into an

electrical signal. This approach thus allows the interaction between ligands and

neurotransmitter systems to be studied in vivo in the brains of patients.
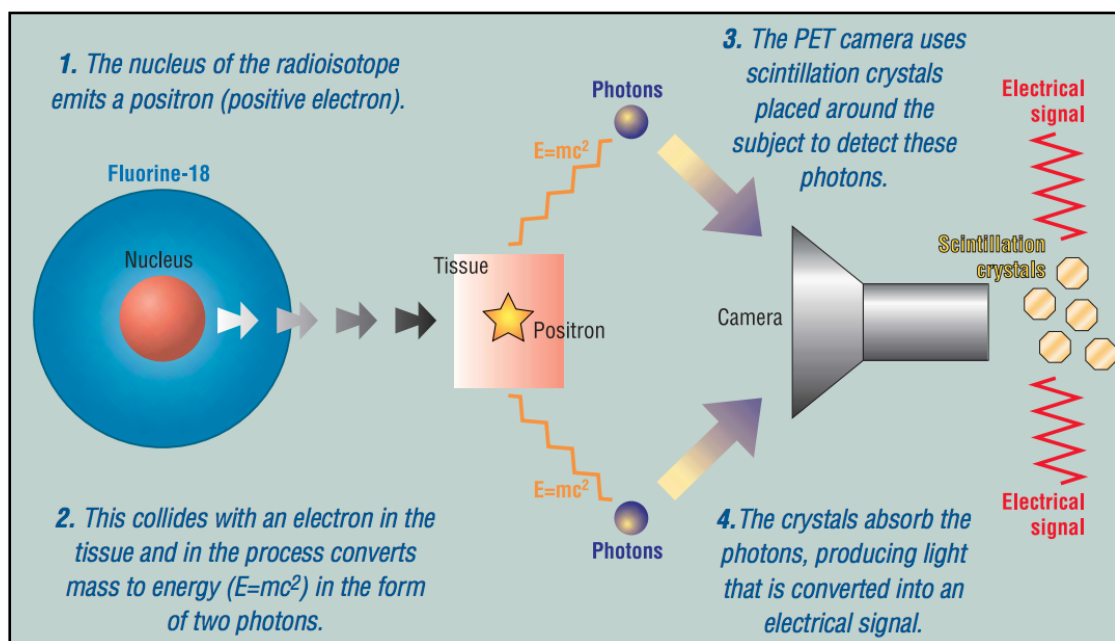
Figure 6: Positron Emission Tomography – How Does it Work (from Berger (2003) [305]).

1.5.1.1.   Dopamine and Positron Emission Tomography Imaging

L-DOPA is an amino acid that contains two hydroxyl groups on the third and fourth positions of the phenol ring. It can be labelled with the positron emitter isotope ^{18}F in the sixth position, forming ^{18}F-DOPA, thus allowing PET imaging of receptors [305]. L-DOPA is the precursor of the neurotransmitters dopamine, norepinephrine (noradrenaline) and epinephrine (adrenaline), collectively called catecholamines. ^{18}F-DOPA is a large neutral amino acid that resembles natural L-DOPA biochemically, has similar kinetics and enters the catecholamine metabolic pathway of endogenous L-DOPA in the brain [306]. PET studies, using [^{18}F]DOPA and [^{11}C]L-DOPA ligands, have investigated the nature of dopamine dysfunction in schizophrenia. Radiolabelling of the dopamine precursor L-dihydroxyphenylalanine allows the measurement of its uptake and conversion to dopamine in dopamine neurons to provide an index of dopamine synthesis capacity.

1.5.2.   Functional Magnetic Resonance Imaging

1.5.2.1.   Background

Functional magnetic resonance imaging (fMRI) was first developed during the 1990s to measure regional, time-varying variations in brain metabolism linked with brain activation during a task [307]. It is a primary tool for basic studies of the organisation of working memory in humans and is a non-invasive imaging technique with good spatial (millimetres) and temporal (seconds) resolution [308].

1.5.2.2.   How Magnetic Resonance Imaging (MRI) works

The human body is made up of about 70% water (H_2O), and therefore the body has many protons in the form of hydrogen nuclei. Protons are electrically charged and because of this, they rotate on an axis. When a person lies in the MRI scanner, the magnetic field in the

scanner makes hydrogen nuclei available in body water align with the field strength (B_0);

either parallel or anti-parallel. They also precess due to magnetic momentum of the atom at

the Larmor frequency:

$$\omega_0 = \gamma B_0 \qquad \omega_0 = \text{Precessional or Larmor frequency. (MHz)}$$

γ = Gyro Magnetic Ratio. (MHz/T)

B_0= Magnetic field strength. (T)

The Larmor frequency is used to calculate the operating frequency of the MRI system.

Overall, there is a net magnetisation, the sum of all of the magnetic field of each proton,

pointing in the same direction as the system's magnetic field. Figure 7 provides an overview

of the basic process of fMRI image acquisition and analysis:

Excitation → Relaxation → Acquisition → Computing → Display
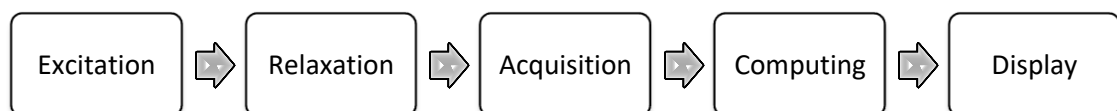
Figure 7: Stages required to obtain an MRI image

A radio frequency (RF) pulse is used to excite the H_1 protons, taking them from a lower

energy state to a higher energy state. When B_0 is turned off (relaxation), the protons return

to their lower energy state. The scanner detects this production of an "echo"

(electromagnetic signal) that is detected via a receiver coil (acquisition). The receiver coil is

placed near the head. The protons want to go back to equilibrium and do so by releasing the

absorbed energy as RF waves.

The relaxation process can be divided into T1 relaxation and T2 relaxation. The net

magnetisation rotates back to align itself with the Z-axis (T1 relaxation). The process of

getting all protons from a total in-phase situation (where the all spin in the same direction at

the same time) to a total out-of-phase situation (where they spin at different times but still

on the x-y axis) is called T2 relaxation. An image can be created as protons in different tissues

have different T2 relaxation times. The RF pulse sequence can be used to make contrast

between different tissue types (in structural imaging) or as a contrast between other

properties (in functional imaging). In fMRI, the scanner is adjusted to resonate as with a

conventional MRI but with different T2* relaxation times. MRI uses a large magnet with a

field strength (B_0) between 1.5 to 7 Tesla.

1.5.2.3.   BOLD-fMRI

Blood Oxygen Level Dependent (BOLD) contrast [309] is a common fMRI technique. It maps

hemodynamic response related to neuronal activity in the brain. Neural activation leads to

an increased demand for oxygen and cerebral blood flow. Neurovascular coupling defines

the basic relationship between increased blood flow and neuronal activity. With intact

neurovascular coupling, a coordinated procession happens from neuronal mass action to

changes in cerebral blood flow, and blood volume leading to increased oxygenated

haemoglobin and subsequent elevation in BOLD signal [310, 311]. Astrocytes also have a role

in neurovascular coupling [312]. Astrocytes release potassium, epoxyeicosatrienoic acids and

prostaglandins in response to neural activity, which all adds to vasodilation of neighbouring

arterioles [313, 314].

The vascular system provides too much oxygenated blood in response to deoxygenation [315], thereby increasing the volume of oxygenated haemoglobin in relation to deoxygenated haemoglobin. Oxygenated and deoxygenated haemoglobin have different magnetic properties; the former is magnetically weaker and, the latter is paramagnetic and lowers fMRI signal. This elevated concentration of diamagnetic oxygenated haemoglobin and reduced field distortions from paramagnetic deoxyhaemoglobin creates the elevation in BOLD signal contrast. These changing concentrations of deoxyhaemoglobin and oxyhaemoglobin make up the basis of the BOLD effect [316].

1.5.2.4.   Task Design in Functional Imaging

Repeated acquisitions help detect significant changes in the signal-to-noise ratio at a voxel or region of interest level. The behavioural task chosen needs to maximise task-related signal changes. There are three types of tasks used in fMRI: block, event or mixed. Block design produces the strongest task-related fMRI signal and is used in the experimental chapters in this thesis. Block designs are divided into experimental and control conditions containing alternating blocks of, for example 30 seconds, per trail during the scan.

1.5.3.   Combining fMRI and PET

Working memory function is regulated by a large neural network that includes the prefrontal and parietal cortex [317, 318], along with support from the dorsolateral prefrontal regions [318]. Pharmacological manipulation of dopamine activity in animals [164, 165] and healthy subjects [319] during working memory tasks alters prefrontal function at both the regional and neuronal level. Meanwhile, dopamine agonists can modulate task-dependent neural activity in the prefrontal cortex [320, 321] and enhance working memory performance [322]. Schizophrenia is linked with over-activity of the subcortical dopaminergic system. This leads

to increased dopamine activity and release in the striatum, as well as the development of psychotic symptoms [111, 323].

There have been three previous studies in chronic and at-risk patients that have looked at the relationship between striatal dopamine synthesis capacity and prefrontal cortex dysfunction. At risk patients have been defined as having a significantly increased clinical risk of becoming psychotic, known as an 'At-Risk Mental State' [324]. Two of these studies have shown a link between greater striatal dopamine synthesis capacity and reduced prefrontal cortex activation during a working memory task in the at-risk population [139, 140]. However, not all patients who are at ultra-high risk do develop psychosis (transition) [325]. The only study in patients with a psychotic disorder was a study of six chronic patients with schizophrenia and used an executive function task (Wisconsin Card Sorting Task (WCST)). It found higher striatal dopamine uptake was significantly associated with lower PFC activation levels in the patient group, but not controls [326]. Though working memory is important for successful performance in the WCST, there are several other cognitive functions necessary to perform the WCST, such as deducing a rule, planning, inhibition etc. Therefore, it is not clear whether poor performance from patients in the WCST results from working memory deficits or another cognitive dysfunction. The key gap in the literature is that there are no studies combining fMRI and PET to look at working memory and striatal dopamine in first episode psychosis patients.

1.6. Structural, Functional and Physiological Differences Observed by the Extant Literature

In this section, I will examine at evidence of structural, functional, and physiological differences in the existing literature. I will look at structural imaging focusing on the

hippocampus, thalamus, and dorsolateral prefrontal cortex evidence from magnetic resonance imaging papers. With regards to functional differences, I will look at the fMRI literature focusing on executive function, working memory and negative symptoms in the DLPFC, thalamus and hippocampus. I will also present a PRISMA literature review of the N-Back working memory task in people with schizophrenia. Finally, I will explore the physiological differences, particularly looking at dopamine and its relationship with working memory and its relationship with the DLPFC.

1.6.1.   Structural Differences (DLPFC, Thalamus and Hippocampus)

1.6.1.1.   Dorsolateral Prefrontal Cortex (DLPFC)

The dorsolateral prefrontal cortex (DLPFC) is engaged in working memory and executive functions, such as task switching and task-set reconfiguration, prevention of interference, inhibition, and planning [327-330].   In working memory, the DLPFC is thought to be involved in monitoring and manipulating items [331-336], as well as the maintenance and retrieval of information [337]. Structural neuroimaging studies offer data suggesting that, anatomically, working memory is affected by schizophrenia in this region [338-344]. It has been reported that there is a greater reduction in temporal and frontal cortical volumes compared with posterior cortical volumes in patients with schizophrenia compared to their siblings or healthy controls [345]. After considering individual differences in gyri patterning and shape, the DLPFC is an important cortical region in which grey matter is reduced in volume in patients with schizophrenia compared with their unaffected monozygotic twins. These are changes that can be correlated with the degree of cognitive dysfunction and negative symptom severity in the patients [33]. Compared to healthy controls, patients with schizophrenia had reductions in cortical thickness in frontal and temporal brain regions. Wheeler et al (2014) found that participants with relatively poorer working memory performance showed a

significantly stronger correlation between cortical thickness in the left and right DLPFC. It was also found that people with schizophrenia had poor working memory performance which was associated with strong left-right DLPFC correlations. This relationship between left-right DLPFC thickness correlations and working memory (WM) performance suggests that prefrontal interhemispheric circuit impairment is a vulnerability pathway for poor working memory performance [346].

1.6.1.2.   Thalamus

The thalamus is believed to contribute cognitive processes, including verbal fluency [347], attention, speed of information processing, memory [348] and working memory [349-351]. Lesion studies have indicated that the thalamus is engaged in memory processing, with memory deficits being seen in patients following thalamic lesions [352, 353]. It is a critical node in the thalamo-cortico basal-ganglian circuitry and inputs a third of all afferent projections into the striatum. The thalamus is thought to play an important role in the pathophysiology of schizophrenia in part owing to its unique location and connectivity. The thalamus acts as a central relay station, transferring peripheral sensory inputs to the cortex and mediating corticocortical connections between areas particularly implicated in schizophrenia, such as frontal and temporal cortices. Some theoretical explanations of schizophrenia have placed the thalamus at the centre of dysfunctional neural connectivity [354].

Structural neuroimaging studies of the thalamus have provided evidence of volumetric, planimetric, or morphologic irregularities in people with schizophrenia [343, 355-359]. Specific grey matter volume reductions have been seen in the thalamus [360]. Family studies suggest that this abnormality might be in part genetically mediated [361, 362]. First episode

patients showed decreased brain grey matter volumes in the thalamus compared with healthy controls [363]. Although some studies have not found evidence of area or volume reductions [364-367] (for a review, please see Sim et al [368]). Recent neuroimaging studies pointed to a selective decrease in anterior and mediodorsal thalamic subnuclei [357, 369]. Post-mortem studies have also observed reductions in neuron numbers and volume in the thalamus in schizophrenia, especially in the mediodorsal nucleus [370-374]. Not all post-mortem studies, however, have shown mediodorsal abnormalities [375]. In non-human primates, studies suggest that the mediodorsal nucleus of the thalamus might transmit prospective motor information to the dorsolateral PFC [376, 377]. Given the major interconnections between the mediodorsal thalamic nucleus and the prefrontal cortex, it might be possible that the genetic contribution to frontal lobe abnormalities in schizophrenia [33] may be reflected in the structure of the thalamus.

1.6.1.3.   Hippocampus

The hippocampus, rich in dopamine receptors [378], is important in the storage of memories and formation of associations [379], encoding, novelty detection and successful retrieval [380, 381]. As discussed earlier, these cognitive processes are involved in working memory tasks. Hippocampal anatomical structural differences have been demonstrated in imaging and post-mortem studies of schizophrenia patients [382]. Decreased hippocampal volume in patients with schizophrenia has been presented [383-385]. Compared with healthy controls, patients with schizophrenia had smaller hippocampus, thalamus, and intracranial volumes [386]. MRI studies show a decrease in hippocampal size (see meta-analytical reviews in [385, 387, 388]) and altered hippocampal shape [389]. Smaller hippocampal volumes have been seen in both prodromal and first episode participants [390-393] showing that hippocampal engagement is not ancillary to the illness or its treatment. Twin studies suggest that smaller

hippocampal size might also be part of the underlying genetic predisposition to schizophrenia [394].

In schizophrenia, the hippocampus and DLPFC were most consistently implicated [395]. The association of frontal lobe and medial temporal lobe structural brain abnormalities with cognitive deficits in schizophrenia, had led researchers to hypothesize that dysfunction in this network might be core to pathophysiology of the illness [234]. The biological plausibility for this network is emphasised by anatomic tracing studies in non-human primates and rodent including the anterior medial temporal lobe to the medial frontal lobe [396]. The projection between the hippocampus and prefrontal cortex contains a bidirectional polysynaptic pathway connecting the DLPFC to the posterior hippocampus via the parahippocampal gyrus but is not part of the anterior hippocampal – medial frontal network [233].

1.6.2. Evidence from Structural Imaging Studies in Working Memory and Negative Symptoms

The severity of negative symptoms and cognitive impairments is highly predictive of a patient's long term prognosis in schizophrenia [176]. Unlike positive symptoms, cognitive and negative symptoms remain stable over time [255], respond poorly to antipsychotic medication [252], are good predictors of functional outcome [397] and both are linked with dysfunction in similar brain regions [398]. Working memory impairment has been directly linked to the presence of negative symptoms [399] or increased symptom severity in patients with schizophrenia [400].

Structural imaging studies have demonstrated that a loss of frontal brain matter is linked to the severity of negative symptoms [340, 342, 401] and cognitive dysfunction including a

reduction in working memory [344, 402] in schizophrenia. Negative symptoms are associated with structural abnormalities in the frontal cortex during working memory tasks [403]. Patients with the most severe negative symptoms also presented with the greatest working memory deficits ([404-406]). Performance on working memory tasks has been associated with negative symptoms [400].

1.6.3. Functional Differences (Executive Function and Working Memory)

1.6.3.1. Executive Function

Studies in healthy volunteers have shown that tasks involving executive function engage the DLPFC [407]. Studies in medicated, treatment-free and treatment-naïve patients with schizophrenia show alterations in DLPFC activation during executive tasks, with some showing hypoactivation in the DLPFC, defined as Brodmann areas 9 and 46, [188, 222, 326, 408-435], and others showing hyperactivation [436-440]. It has been suggested that the inconsistency in the direction of activation is due to performance effects: studies did not control for performance difference but did find that those patients who performed better had hyperactivation whilst those who performed poorly had hypoactivation of the DLPFC [436, 437, 441, 442]. Executive function and working memory (WM) impairment are common cognitive deficits in schizophrenia [400, 443, 444] particularly from illness onset [445-447]. There is thus a need to understand the neurobiology underlying cognitive impairments to guide the development of treatments.

1.6.3.2. Working Memory and N-Back Task

Working memory (WM), the capacity to retain information in mind for short periods of time, is a key cognitive function required for many day-to-day tasks [448]. Previously, the terms

working memory and short-term memory have been used synonymously [449]. Short-term

memory is confined to a somewhat passive storage of material, whilst working memory

includes the use of information online. Engle et al (1999) suggested that short-term memory

should refer to the simple storing of information, whilst working memory should include the

notion of content manipulation [450].

Working memory is commonly impaired in schizophrenia [400, 443, 444] and is characterised

by a reduction in accuracy and an increase in response time. This has been evidenced in

various stages of the illness including ultra-high risk (UHR) (see meta-analysis [451], illness

onset [445-447], patients with their first-episode of schizophrenia (see meta-analysis [452])

and in chronic patients [453]. Many neuroimaging findings indicate that regions involving the

dorsal frontal-parietal network are altered in schizophrenia and it is this neuropathology that

might underlie working memory impairments in schizophrenia [454]. It has furthermore been

suggested that the inverted U–shaped relation of DLPFC activation to working memory loads

is shifted to the left in patients with schizophrenia compared to healthy controls [414, 427,

454, 455]. Comparable irregular brain activation is also seen in the dorsal parietal cortex in

schizophrenia indicating frontal-parietal dysfunction underlying higher cognitive loads during

working memory [456].

It is thought that there is a reduced ability to access information stored in the hippocampus

during a memory recall task in patients [230, 239]. Poor performance on working memory

and executive function tasks might be related to impairments in retrieval, storage and

maintenance functions [457] in the DLPFC and related to hypoactivation in the hippocampus

[458] in the latter. There is also evidence linking cortical brain function with negative

symptoms in schizophrenia. Lower activation levels during a WM task in the left parietal and

frontal regions were related to greater negative symptoms [459]. An inverse correlation

between negative symptoms and cognitive performance was also seen in a higher working

memory load of the N-Back task [460]. DLPFC activation correlated negatively with working

memory performance in both first episode psychosis patients and patients with chronic

schizophrenia [461]. In first episode patients with schizophrenia, the amount of improvement

in executive function and working memory is significantly related to a reduction in negative

symptoms [462]. However, whilst increased activation in DLPFC has been associated with

improvement in negative symptoms, patients whose negative symptoms improved showed

no difference in WM performance from those who did not [295], suggesting that the neural

basis of negative symptoms and WM impairments may be different.

The N-Back task has been used to investigate the neural basis of working memory and reliably

activates a network of brain regions that include the dorsolateral prefrontal cortex (DLPFC),

hippocampus, thalamus and other subcortical regions [318, 463-465] in healthy participants

(see [318] for a meta-analysis). During the N-Back task, participants are asked to monitor the

identity of a series of letters and to indicate when the currently presented stimulus was the

same as the one presented *n* trials previously; where *n* is 0, 1 or 2. 1-Back and 2-Back are the

experimental conditions and 0-Back is the control condition. The subtraction paradigm [466]

is where activation during the experimental condition is subtracted from the control

condition to identify areas and functions of interest. Figure 8 shows examples of each

condition. The order of difficulty increases from 0-back (control condition) to 1-Back to 2-

Back (most difficult).
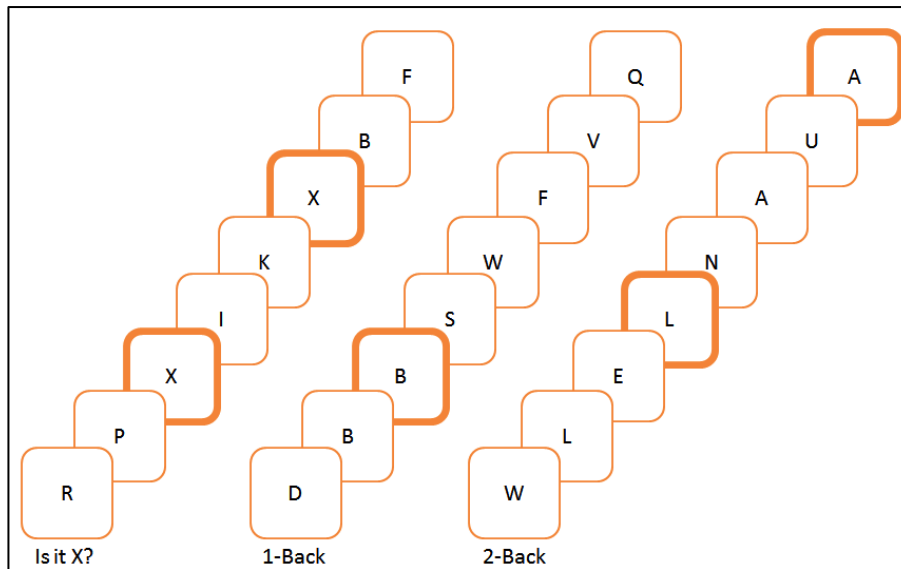
Figure 8: N-Back Task with 0-Back (is it X?), 1-Back and 2-Back conditions. Targets are indicated with thick-edged boxes for each condition.

1.6.3.2.1. Cognitive Processes in N-Back Working Memory Task

I will now give an account of the cognitive processes involved in N-Back performance over and above the maintenance of information in working memory, along with processes that contribute to the 1-back and 2-back conditions.

Jonides et al. (1997) [467] detailed seven processes that were needed for successful N-Back performance:

1. Encoding each stimulus

2. Storing any stimulus relevant to a future decision

3. Rehearsing to keep the items in storage active and ready.

4. Matching when comparing the present item to previous item(s)

5. Temporal ordering to know which item should be used for comparison to the present item.

6. Inhibiting the oldest item to replace with the newest item in the series.

7. Executing the response.

As discussed above earlier in the introduction, the N-Back involves seven cognitive processes. First the N-Back task requires the encoding and temporary storage of each "n" item in the sequence, and then the continuous updating of the store with new incoming stimuli. In parallel, no longer relevant stimuli have to be inhibited and abandoned from working memory. Additionally, a matching and counting process between the stored and upcoming stimulus is necessary to make a decision as to whether the stimuli are the same thus initiating a correct response [468, 469]. There are qualitative differences between 2-back and 1-back. The 2-back condition requires longer storage duration of the stimuli and more processing time than 1-back because participants must compare each stimulus to the item that was seen or heard two items ago. Additionally, the 2 back requires additional cognitive resources for retaining each stimulus for future comparisons and constantly updating memory to include only the two most recent items. Finally, the 2-back condition requires temporal ordering of stimuli, which is not required in the 1-back condition.

Although the storage and rehearsal demands of the 1-back task are reduced relative to the 2-back condition, it still requires working memory to retain the stimuli [470]. Studies generally report linear patterns in DLPFC activation as the memory load increases from 0 to 3 back (e.g., [467, 471]). However, a study using functional MRI reported almost no difference from 0 to 1 back or 2 to 3 back in activation in the DLPFC but a large increase in activation from 1 to 2 back. This indicates that the DLPFC is particularly engaged by the increased memory load from the 1-back to 2-back conditions [472]. It has been suggested, based on the evidence

from this paper, that the contrast between the 1-back and 2-back conditions is, thus, a useful index of DLPFC function [473].

1.6.3.3. N-Back Task in Schizophrenia

Below is a PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) checklist and flow diagram to illustrate how the literature review was conducted. The PRISMA Checklist provides a list of elements that should be included in the review manuscript. The PRISMA Flow Diagram provides a standard means of illustrating the review process.

1.6.3.4. PRISMA Checklist

1.6.3.4.1. Title

A literature review of N-Back Working Memory fMRI studies of First Episode Patients with Psychosis.

1.6.3.4.2. Abstract

1.6.3.4.2.1. Structured Summary

Working memory can be impaired in patients with schizophrenia. The objective of this review is to systematically review and summarise the findings of studies using N-Back working memory task and fMRI in patients with first episode psychosis. PubMed and Google Scholar were the data sources. The study eligibility criteria were that papers had to include the N-Back working memory paradigm during fMRI. The participants were patients with first episode psychosis and healthy volunteers. The intervention was the N-Back task. The results in patients were largely consistent. In first episode patients (see Table 2) compared to

healthy controls, there is hypoactivation in the dorsolateral prefrontal cortex (DLPFC) [166, 167, 344], thalamus [344]187] and hippocampus [345]. The main limitation was that only 2 search engines were used. There is an implication that worse N-Back behavioural performance in patients compared to controls might be related to impairments in retrieval, storage, and maintenance functions [296]. There is no systematic review registration number as this literature review is published as part of a PhD thesis and was not independently registered.

1.6.3.4.3. Introduction

1.6.3.4.3.1. Rationale

Working memory in patients at risk of developing schizophrenia, in patients with chronic schizophrenia and in patients with first episode psychosis is often impaired. The N-Back working memory task is a useful tool for measuring working memory.

1.6.3.4.3.2. Objectives

The objective of this literature review is to systematically review and summarise the findings of studies using N-Back working memory task and fMRI in patients with first episode psychosis.

1.6.3.4.4. Methods

1.6.3.4.4.1. Protocol and registration

A protocol was not produced and was not registered as this literature review is part of a thesis and not a publication. This is in line with the KCL guidance for a PhD thesis. KCL does not require using PRISMA guidelines for PhD dissertations.

1.6.3.4.4.2. Eligibility criteria

The specific study criteria were patients with first episode psychosis, N-Back task (intervention), compared to healthy volunteers. The outcomes were the activation measured during the N-Back task during the fMRI scan.

1.6.3.4.4.3. Information sources

PubMed and Google Scholar were the two databases used in the search. The date of the first search was 1st september2017. The date of the last search was 21st September 2020.

1.6.3.4.4.4. Search

The search terms for my literature review were: "First Episode Schizophrenia or First Episode Psychosis" AND "fMRI".

1.6.3.4.4.5. Study selection

Participants of all ages, genders, races, and ethnicities were included. Articles were included in this literature review if they met the following criteria: (a) written in English; (b) reported as original research; and (c) reported using N-Back and fMRI. Studies were excluded if they: (a) contained editorials, book reviews, or opinion pieces; (b) contained genetic research; (c) included only chronic patients. Nine studies were included in the review.

1.6.3.4.4.6. Data collection process

Data was extracted independently from reports. A table was used, populated with data from the literature search. The process for selecting studies once the search terms had been entered was firstly to read all the tiles to see if they were applicable to the literature

research. Secondly, the abstracts were read to either confirm or investigate further if the papers were eligible for this review. Thirdly, if it was not clear from the abstract, the methods section was looked at to see if the N-Back working memory task and fMRI scans were used in patients with their first episode of psychosis in the paper in question. Any articles that did not meet these criteria were excluded.

1.6.3.4.4.7. Data items

Data was from the articles was compiled into Table 1 below under the following headings:

Study which referred to the name of the study.

Treated which referred to whether the patients included in the study were already on medication to alleviate their schizophrenia symptoms.

Performance controlled which refers to whether performance on the N-Back working memory task as controlled for thus ensuring participants understood the task correctly;

Sample Size (FEP/HC) refers to the number of participants included in the paper; FEP activation refers to any activation differences between the groups that the studies found.

Main Finding – this refers to the main findings of the paper as reported by the authors and whether statistics were significant.

1.6.3.4.4.8. Risk of bias in individual studies

The following methods were used to assess the risk of bias of individual studies, and this was done at the outcome level. The sample size was looked as this is an important confounding factor as the study might be underpowered and a type I error may have occurred whereby the null hypothesis was rejected. Another factor that was looked at was inclusion of

medicated patients as there is believed to be an effect of antipsychotic medication on BOLD signal.

1.6.3.4.4.9. Summary measures

The principal summary measure was the areas of activation during the N-Back working memory in patients with their first episode of psychosis compared.

1.6.3.4.4.10. Synthesis of results

The main findings and other factors (listed above) from each study was entered into Figure 9 below. The results of the studies were not combined.

1.6.3.4.4.11. Risk of bias across studies

Only two databases were used, PubMed and Google Scholar, so there may have been an effect on the cumulative evidence which might have been diminished with more than two databases used. I did not assess bias across the studies for this literature review.

1.6.3.4.4.12. Additional analyses

No additional analyses were used in this review.

1.6.3.4.5. Results
1.6.3.4.5.1. Study selection

The number of studies screened, assessed for eligibility, and included in the review are below in the flow chart (Figure 9). The reasons for excluding studies once the duplicates had been

removed were: the working memory task used was not the N-Back task; no fMRI was used;

the population did not include patients with their first episode of psychosis.

1.6.3.4.5.2. Study characteristics
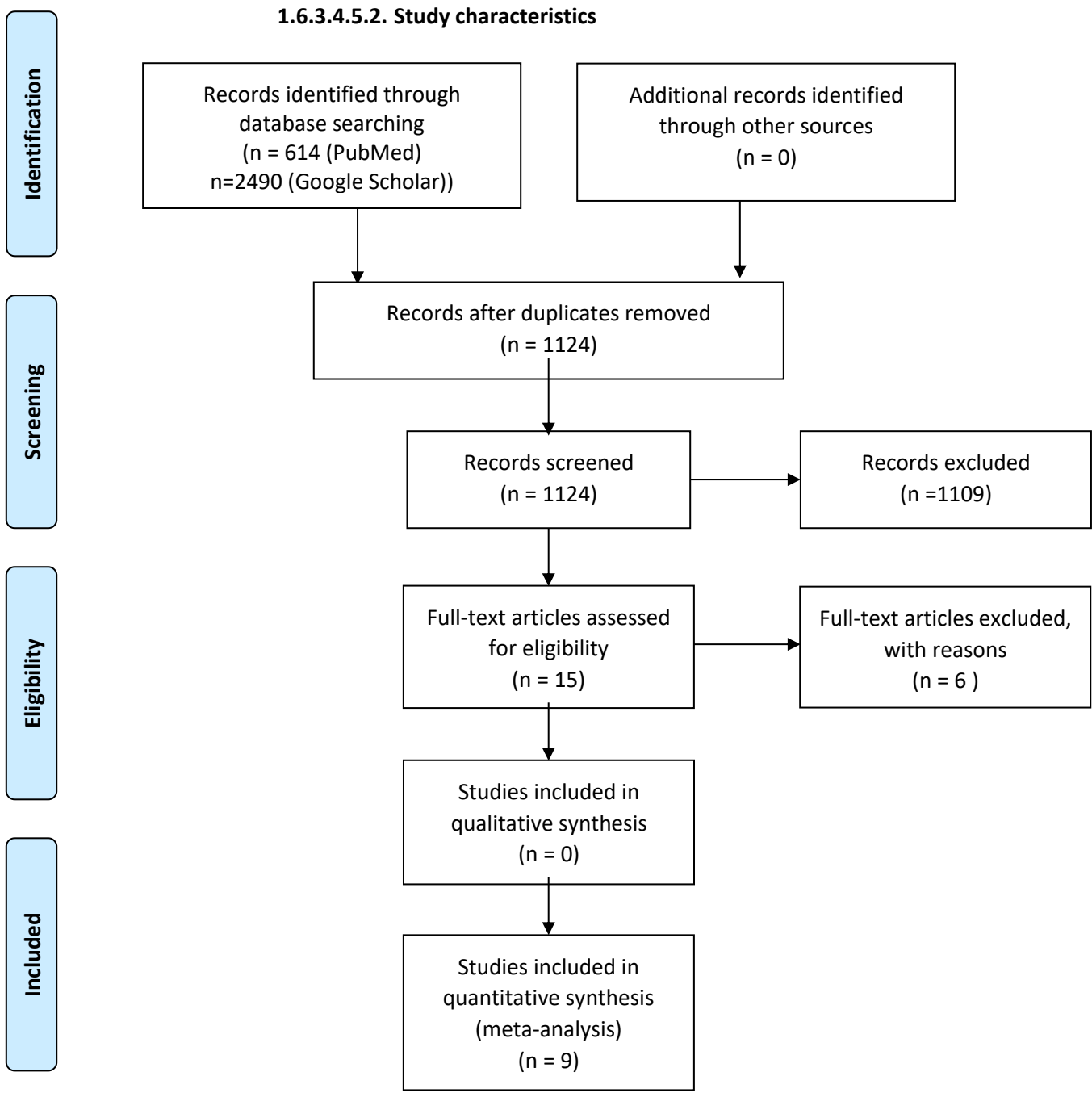
Figure 9: PRISMA 2009 Flow Diagram

The sample sizes ranged from the smallest with 18 total sample (10 patients with FEP) to the largest with 156 (75 patients with FEP). Behavioural performance was controlled for in three of the nine studies, as follows:

- excluding participants with less than a 90% hit rate or total number of correct trials, or having more than four missed responses in the 0-back control condition of the N-Back task [474].

- Reaction times to incorrect responses were excluded. [296]

- Using the signal detection theory index of sensitivity, d', to exclude subjects who were unlikely to be performing the task [475]. Participants who had negative d' values in either or both 1-back and 2-back versions of the task, which suggested that they were not performing the task, were a priori excluded from the study. [476]

- However, behavioural performance was controlled for in other studies. For example, in Vogel et al (2016) [461], the sensitivity index d' was calculated to quantify task performance by using the formula d' = z(Hits) − z(False Alarms [477]). Hit and false alarm rates of zero or 1 were adjusted as previously described [477]. Vogel et al found significantly poorer WM performance in SZ patients compared to the FEP group, but did not exclude poorly performing subjects, or adjusted for differences in performance between groups. This significant difference between groups could be explained by performance differences and participants not performing the tasks properly or misunderstanding the task.

1.6.3.4.5.3. Different Analytic Approaches

Several different analytic approaches were used in the studies, including linear contrasts, two-sample and paired t-tests, differences on regression slopes, correlations, and linear

regression. The next two paragraphs provide further details of these tests for each of the studies listed in the literature review table (Table 2). To test for group differences, Nejad et al. (2011) [474] specified linear contrasts to model main group and interaction effects within the N-Back conditions. They compared the 2-back WM condition relative to the 1-back WM condition, which was relative to the 0-back WM condition. In Mendrek et al (2004)'s study [294] the differences between groups in the 2-back and 0-back contrast, and between 0-back and rest condition in each session were analyzed using two-sample t-tests. Meisenzahl et al. (2006) [296] used two-sample t-tests for intergroup and paired t-tests for intragroup (before and after treatment) comparisons of the patients' data. Between-group comparisons were performed on 2-back with the 0-back condition (working memory) contrasts using two-sample t-tests in Schneider et al. (2007) [457]'s paper. In Guerrero-Pedraza et al. (2012) [476]'s study, group comparisons between patients and controls were performed within the FEAT module (included within FSL), with mixed effects GLMs [478]. They also examined the effect of increasing working memory load on the differences between patients and controls. They did this by fitting models that assume a linear relationship between activation and N-Back condition from the baseline and 1-back to the 2-back levels of the task and reported significant differences on regression slopes between the two groups.

To specify the WM-associated network of activation, Smieskova et al (2012) [479] first identified the main-effect of the n-back task (using a full-factorial model with $P < 0.001$, FWE-corrected, to identify regions significantly activated by the task), and then used this as a mask for the 2nd level analyses to identify regions were activation differed between groups. The correlation between BOLD signal and grey matter volume (GMV) was calculated on a voxel-by-voxel basis with the biological parametric mapping (BPM) correlation model [480]. In Vogel et al. (2016) [355]'s study, between-group differences were evaluated by using the 2-

sample t-test. The groups differed with respect to age, so this variable was added as covariate in the second level analysis. In the Crossley et al. (2009) [481] paper, an event-related analysis was performed on the block-design-acquired data. A general linear model was used to calculate the parameter estimates for all brain voxels. A second level analysis was performed using the pooled 1- and 2- back contrasts to identify areas activated during the working memory task, independent of mnemonic load (with a threshold of $P < 0.05$, corrected with FWE with clusters less than 10 voxels and masked with contrasts of activated regions for each independent group at $P < 0.001$). Differences in activation between the three groups were investigated using a statistical threshold of $P < 0.05$, corrected with FWE and an extent threshold of 10 voxels. For their *a priori* ROI, the superior temporal cortex, group-related differences were found using a statistical threshold of $P < 0.001$ and an extent threshold of 10 voxels. Finally, in Nielsen et al. (2017) [482], they used linear regression to explore brain–behavior relationships.

Each study used different analytic approaches, with some overlap between papers. The two studies that had hyperactivation [481] or trend-level activation [474], compared to the majority that showed hypofunction, used either a pooled 1 and 2 back contrasts [481] or linear contrasts. Pooled contrasts might have ignored the effect on activation of each level of difficulty of the task. Linear contrasts enables one to express the sum of squared differences between the means of sets of treatments, but the formula does assume equal sample sizes, which, was not the case in the study by Nejad et al [474], (23 FEP and 35 healthy controls).

1.6.3.4.5.4. Risk of bias within studies

Below, I will detail the risk of bias of each study. Outcome level assessments were not available. In Nejad et al. (2011)'s study, the groups were not matched on the parental

socioeconomic measure and this might be a potential risk of bias because evidence suggests that there is a correlation between socioeconomic status and cognitive development [483]. Mendrek et al (2004) [294]'s had the smallest sample of the nine studies, used medicated patients and did not control for performance leading to an underpowered sample with skewed results from the effect of the medication and potentially participants not understanding the task correctly. Meisenzahl et al. (2006) [296]'s study used a degraded 2-back Vs 0-back where the screen was blurred perhaps making the task more attentional than working memory owing to concentration required. Schneider et al. (2007) [457]'s paper used treated patients and did not control for performance both of which may have been sources of bias. Guerrero-Pedraza et al. (2012) [476] also looked at patients who were on treatment but did control for performance. Smieskova et al (2012) [479] also included medicated patients and did not control for performance. Vogel et al. (2016) [461] used treated patients but did control for performance. Crossley et al. (2009) [481] also included treated patients. In addition to this, in their first level analysis they used "non-target" (rather than target) events for their event-related analysis as they were not linked with motor responses. In doing so, however, they did not accurately report the results from the contrasts as the other studies did and this bias might be the reason for the increase in activation that they found, inversely to all the other studies in this literature review. Lastly, Nielsen et al. (2017) [482] included treated patients and did not control for performance.

1.6.3.4.5.5. Results of individual studies

I will now provide a simple summary for each intervention group. In first episode patients (see Table 2) compared to healthy controls, there is hypoactivation in the dorsolateral prefrontal cortex (DLPFC) [294, 296, 461, 479, 482], thalamus [294, 296] and hippocampus [476]. There have also been reported trend level hyperactivation in the DLPFC in first episode

treatment-naïve patients [474]. Only one study found significant hyperactivation in first

episode patients in the superior temporal cortex compared to controls [481], but the sample

size was small, and the patients were already on antipsychotic medication.

Seven out of the nine studies found decreased activation in DLPFC, thalamus, cerebellum,

posterior cingulate, ventral lateral PFC, postcentral gyrus, precuneus, gyrus rectus, temporal

poles, superior parietal lobule, middle frontal gyrus and superior frontal gyrus in patients

compared with healthy controls during the N-Back working memory task. Nejad et al. (2011)

[474] showed that DLPFC and superior parietal cortex trend but failed to reach significance

criterion. In Mendrek et al (2004) [294]'s study, there was decreased activation in the DLPFC,

thalamus, cerebellum and posterior cingulate in patients compared to controls. Meisenzahl

et al. (2006) [296]'s study found decreased activation in the thalamus, right DLPFC and VLPFC.

There was decreased activation in the postcentral gyrus and precuneus in Schneider et al.

(2007) [457]'s paper. Guerrero-Pedraza et al. (2012) [476] found decreased activation in

patients in the gyrus rectus and temporal poles (including hippocampus) compared to

controls. In Smieskova et al (2012) [479]'s study, there was decreased activation in the

precuneus, superior parietal lobule, middle frontal gyrus and superior frontal gyrus. Vogel et

al. (2016) [461] demonstrated that there was decreased activation in the superior frontal

gyrus in patients compared to healthy volunteers. Crossley et al. (2009) [481] was the only

paper to find increased activation in patients compared to healthy controls in the superior

temporal cortex during the N-Back task. Finally, Nielsen et al. (2017) [482] showed that there

was decreased connectivity between the left inferior frontal gyrus and left inferior parietal

lobule modulated by working memory.

1.6.3.4.5.6. Synthesis of results

No meta-analyses were done as part of this literature review.

1.6.3.4.5.7. Risk of bias across studies

These are important confounding factors such as a small sample size which might underpowered a study and might lead to a type II error where there it is declared that there are no differences or associations between study groups when, in fact, there was. With a larger sample size, a different result may have occurred. The second confounding variable is due to effect of the antipsychotic medication on BOLD signal by directly modulating neurons, astrocytes, and cerebral blood flow in addition to further compound the complexity of the biological understanding of the BOLD signal [148, 149]. One final study did not find any difference between patients and controls [345], although this was not due to a small sample size and one can postulate that it might be due to the contrast used (2-Back>0-Back).

1.6.3.4.5.8. Additional analysis

No additional analyses were done.

1.6.3.4.6.   Discussion

1.6.3.4.6.1. Summary of evidence

7 of the 9 papers presented in this literature review demonstrated that there was decreased activation in activation in DLPFC, thalamus, cerebellum, posterior cingulate, ventral lateral PFC, postcentral gyrus, precuneus, gyrus rectus, temporal poles, superior parietal lobule, middle frontal gyrus and superior frontal gyrus in patients compared with healthy controls during the N-Back working memory task. Only two of the studies included untreated or

treatment-naïve patients and only 3 of the 9 papers controlled for performance during the task.

1.6.3.4.6.2. Limitations

As only 2 electronic databases were used, some studies may have been missed. Another limitation was that as this is a PhD thesis which did not have a formal protocol and was not registered, it might have been biased. To improve for the future and publication, a formal protocol and registering the literature review would improve this.

The search terms "dopamine", "striatum" and "PET" were omitted from this literature review as there are already published reviews of schizophrenia, dopamine and working memory [484], as well as reviews of schizophrenia and dopamine (see [107, 113]), neuroimaging studies of the early stages of psychosis [485] and clinical high-risk state for psychosis [486]. Future literature reviews could seek to include these terms and update Tanaka (2006)'s review. Additional terms that could be added for a future literature review could be "working memory deficits" and "symptoms."

1.6.3.4.6.3. Conclusions

I found that fMRI signal in the DLPFC, thalamus, cerebellum, posterior cingulate, ventral lateral PFC, postcentral gyrus, precuneus, gyrus rectus, temporal poles, superior parietal lobule, middle frontal gyrus and superior frontal gyrus lower in patients with psychosis compared to healthy volunteers during performance of a task that involves working memory relative to the control task. Tracing studies show that projections from cortical brain regions to the striatum show a topographical distribution across the striatum, with neurons in the dorsolateral prefrontal cortex predominantly projecting to part of the striatum including the caudate and anterior putamen that is termed the associative striatum [103, 166, 167].

Elevated dopamine synthesis and release capacity has been found in the associative striatum in patients with psychosis [52]. The observation that this striatal region receives projections from the dorsolateral prefrontal cortex [136, 137, 169-171] could suggest an association between altered dorsolateral prefrontal cortex function and dopamine function in the striatum in schizophrenia. I will go on to test this association in my thesis. It should also be recognised that the patients were taking antipsychotic medication in all but 2 of the studies I reviewed, and antipsychotic medication has been found to alter brain function [487]. Thus, it is not possible to exclude a possible confounding effect of antipsychotic treatment in most studies, limiting the conclusions that can be drawn.

In the studies of untreated patients, one did find a significant group difference, but, given the relatively modest sample size, a type II error cannot be excluded [294]. In the other paper of untreated patients, there was a trend level decrease in patients which failed to reach the significance criterion. Thus, I can conclude from my review of studies that it remains unclear if there are significant differences in neural activation during the N-Back task in untreated patients. This indicates that further research with larger samples of untreated patients is needed to investigate if there are significant differences in neural activation between patients and controls free from the potential confound of treatment.

In addition, all studies used the 2-Back>0-Back contrast. As discussed in the introduction (section 1.6.3.2), the 2-back condition involves several cognitive processes, in addition to working memory, that are not involved in the 0-back condition, notably temporal ordering of stimuli and inhibition of no longer required stimuli. This potentially confounds interpretation of the imaging results in terms of working memory. Thus, a more parsimonious interpretation of these findings is that there is altered neural activation in schizophrenia during a cognitive

task involving working memory, temporal ordering of stimuli and inhibition of redundant information. It could be suggested that, in untreated patients, using 2-Back>1-Back in place of 2-Back>0-Back would be a better contrast to test working memory function as the 1-Back also includes temporal ordering and inhibition of redundant information.

Notwithstanding the issues discussed above, the overall pattern of findings I observed in my literature review indicates there is lower neural activation in the DLPFC in patients with schizophrenia during a cognitive task. This is consistent with the central hypothesis of the thesis that there is altered cortical function in schizophrenia during cognitive function. My literature review also highlights the need for further, larger studies in patients free from the potential confound of antipsychotic treatment to exclude the possibility that these alterations are due to antipsychotic effects on brain function. I aim to address this in my thesis by conducting a study in patients with schizophrenia who are not taking antipsychotics. My literature review also identified that the effect of antipsychotic treatment on neural function remains unclear. My thesis aims to investigate this as well.

1.6.3.4.7. Funding

Table 1: PRISMA Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	75
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number	75
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	76
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	76

Section/topic	#	Checklist item	Reported on page #
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	76
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	77
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	77
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	77
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	77
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in	77

Section/topic	#	Checklist item	Reported on page #
		duplicate) and any processes for obtaining and confirming data from investigators.	
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	78
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	78
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	79
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	79
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	79
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	79

Section/topic	#	Checklist item	Reported on page #
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	79
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	80
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	83
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	84
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	86
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	86

Section/topic	#	Checklist item	Reported on page #
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	86
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	86
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	87
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	87
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	89

Table 2: Results from the literature search on N-Back Working Memory fMRI studies of First Episode Patients with Psychosis

Study	Treated	Performance controlled?	Sample Size (FEP/HC)	FEP activation	Contrast	Main Finding
Nejad et al. (2011) [474]	No	Yes	23 / 35	↗	2-Back>0-Back	DLPFC and superior parietal cortex trend increase but failed to reach significance criterion.
Mendrek et al (2004) [294]	Yes	No	10/8	↓	2-Back Vs 0-Back, 0-Back Vs Rest	DLPFC, thalamus, cerebellum and posterior cingulate
Meisenzahl et al. (2006) [296]	No	Yes.	16/12	↓	2-Back>0-Back, 2-Back>0-Back (degraded)	Thalamus, right DLPFC and VLPFC.
Schneider et al. (2007) [457]	Yes	No.	75/81	↓	2-Back Vs 0-Back, 0-Back Vs Baseline	Postcentral gyrus and Precuneus.

Study	Treated	Performance controlled?	Sample Size (FEP/HC)	FEP activation	Contrast	Main Finding
Guerrero-Pedraza et al. (2012) [476]	Yes	Yes	30/28	↓	0-Back Vs 1-Back, 0-Back Vs 2-Back	Gyrus rectus and temporal poles (including hippocampus)
Smieskova et al (2012) [479]	Yes	No	21/20	↓	2-Back>0-Back	Precuneus, superior parietal lobule, MFG and SFG.
Vogel et al. (2016) [461]	Yes	No	22 / 20 (Chronic Scz)	↓	2-Back>0-Back	SFG – correlated positively with negative symptoms.
Crossley et al. (2009) [481]	Yes	No	10/13	↑	1-Back2-Back Vs 0-Back, 1-Back Vs 0-Back, 2-Back Vs 0-Back	FEP>HC in superior temporal cortex
Nielsen et al. (2017) [482]	Yes	No	17/18	↓	2-Back Vs 0-Back	Connectivity between the left IFG and left IPL modulated by WM

1.7. Impaired Cognition, Symptoms and Brain Abnormalities

I will now spend some time discussing the potential three-way relationship between working memory impairment, negative symptoms, and brain abnormality, including DLPFC dysfunction.

Brain imaging studies have investigated the relationship between cognitive impairment, symptoms and brain structure and function in schizophrenia. I will focus on the DLPFC, working memory and negative symptoms. A reason to think that these might be related is the cortical hypodopaminergia theory of schizophrenia. Davis et al (1991) [127] hypothesised that negative symptoms in schizophrenia might be linked with reduced dopamine activity in the prefrontal cortex. Davis et al. (1991) [127] postulated that schizophrenia is typified by abnormally low prefrontal dopamine activity, causing negative and cognitive symptoms, which they hypothesised might lead to excessive dopamine activity in mesolimbic dopamine neurons (causing positive symptoms). Negative symptoms in patients with schizophrenia are comparable to those seen in neurological patients with frontal lobe lesions, who also show cognitive impairments [488, 489], so it has been hypothesised that these symptoms result from frontal lobe dysfunction, particularly in the dorsolateral prefrontal cortex [173, 490, 491]. Thus, cortical dysfunction perhaps due to low dopamine might lead to cognitive impairment and negative symptoms, with both sets of symptoms representing the result of a common underlying process of impaired frontal lobe function.

The three-way relationship between working memory impairment, negative symptoms, and brain abnormality, including DLPFC dysfunction was looked at when Nejad et al. (2013) [295], used the N-Back working memory task. They found that patients whose negative symptoms improved, had a greater activation in the right prefrontal and parietal clusters relative to those patients whose symptoms did not improve, but their findings could not be attributed to WM dysfunction as

negative symptom improvers and non-improvers did not differ on working memory performance. Further, at baseline, there was no relationship between negative symptoms and working memory functional connectivity. This suggests that the neural basis of negative symptoms may be distinct from that underlying working memory performance.

1.8. Summary

Negative symptoms are common in schizophrenia, apparent from first episode, and linked with poor functional outcome. Currently, there is no licensed medication for negative symptoms, signifying the need to better comprehend the mechanisms underlying them. The neural mechanisms underlying negative symptoms in patients are unclear. It has been hypothesized that impairments in cortical function could contribute to the development of negative symptoms. The neural mechanism linking these, however, has not been tested. Therefore, in the first experimental chapter, I aimed to test this using fMRI imaging in first episode antipsychotic-free patients.

1.8.1. I hypothesised that there would be less activation in the DLPFC, thalamus and hippocampus during high working memory load relative to lower load in patients than controls.

1.8.2. I further hypothesised that lower activation in patients would be directly associated with greater severity of negative symptoms.

In schizophrenia, patients have impairments in working memory performance and altered brain activation during working memory tasks and striatal dopamine dysfunction. However, it is unknown if these alterations are related at the onset of the disorder. This relationship has not

been previously tested in patients in their first episode of the illness. The second experimental chapter aims to test this by combining PET and fMRI imaging in first episode antipsychotic-free patients

1.8.3.   I hypothesised that the BOLD response in the antipsychotic-free first episode psychosis patients in the DLPFC, hippocampus and thalamus during the working memory tasks would be associated with greater striatal dopamine synthesis capacity.

Antipsychotic treatment has been associated with some improvement in negative and cognitive symptoms, although it is unclear if this is a specific effect or secondary to improvement in other symptoms [252-254]. Determining whether antipsychotic treatment alters the frontotemporal systems thought to contribute negative and cognitive symptoms would help identify antipsychotic treatment has a specific effect. However, studies of patients taking antipsychotic treatments to date have shown inconsistent effects on frontotemporal activation levels in patients across studies [291, 293, 294, 296, 298]. However, all of these studies are cross-sectional, so it is unclear if the contradictory results are due to differences in in the samples or differences in the effects of antipsychotic treatment. In the third experimental chapter, I aimed to address this issue by testing the effect of antipsychotic treatment on the neural response during the N-Back working memory task with fMRI scans obtained prior to and after treatment. This was done to explore whether antipsychotic treatments affect neural response during the N-Back working memory task, and whether a change in neural response is linked to clinical change.

1.8.4. I hypothesised that antipsychotic treatment would increase the neural activation in DLPFC and that the change will be directly correlated to improvements in negative symptoms.

The research presented in this dissertation is divided into two parts. Part I describes the studies at baseline (before treatment) and the aim was to test two hypotheses looking at dorsolateral prefrontal cortex, thalamic and hippocampal dysfunction correlated with striatal dopamine or negative symptoms. Part II looks at the effect of antipsychotic treatment on negative and cognitive symptoms. In this latter section, patients had been on medication for 4-6 weeks.

1.8.5. Conclusions and Study Rationale

A better understanding of the neural mechanisms underlying cognitive impairment is needed in psychosis. This could help identify new treatment approaches for these symptoms. Recent studies in the at-risk and chronic populations have indicated a link between striatal dopamine synthesis capacity and cortical neural responses during cognitive tasks [139, 140, 144, 326]. However, these findings are in patients who have not, and may not, develop psychosis or in long-term medicated patients and so it is unclear whether cortical activation is related to striatal dopamine in unmedicated first episode psychosis patients. The differences between the groups may reflect differences in cognitive impairment at presentation or represent a longitudinal effect of medication on cognition. Therefore, studies are required to investigate cognitive impairment and its relationship with dopamine in treatment-free patients, as well as a longitudinal study following antipsychotic free patients to determine whether baseline cognitive measures are related to striatal dopamine and are affected by treatment.

1.8.5 Overview of the Data Collection and Study Procedures

An overview of the data collection is provided below before moving on to the experimental chapters. This section will give a clear explanation of the study procedures. The reporting of this study, particularly participant retention and assessment timelines has used CONSORT guidelines (www.consort-statement.org). Each experimental chapter has a CONSORT flowchart in the results section and the CONSORT checklist at the end of each chapter. I will also make clear how the samples in each experimental chapter relate to each other.

One sample was collected for this thesis comprising of 45 patients with first episode of psychosis, who were recruited into the study from early intervention clinics. I recruited participants by attending weekly meetings at the EI clinics and speaking to social workers, nurses, and psychiatrists about which of their patients might be suitable. They would then approach patients on my behalf and, if the patient was interested in the study and happy to be contacted by me, I would then get in contact with the patient to discuss the study and send out information sheets, if appropriate.

33 healthy volunteers were recruited via local advertising and via the snowball technique. In the first experimental chapter, 29 of these patients and 30 healthy controls were included in the final analysis. In the second experimental chapter, out of the total sample of 45 patients and 33 controls, 24 patients and 18 healthy volunteers had had PET scans, and 45 patients and 33 healthy controls had MRI. Of this number, 24 patients and 18 healthy controls had both MRI and PET scans and were included in the analysis. In the final experimental chapter (chapter 4), out of the total sample of 45 patients, 16 patients received treatment and completed follow-up measures to be included in the final analysis.

2. **The Neural Basis of Working Memory Impairment in Psychosis and Its Relationship to Negative Symptoms: An fMRI Study in First Episode Patients**

2.1. Abstract

Aim: To explore if working memory load-related activity is reduced in drug free first episode patients, and its association with negative symptom severity.

Method: 29 antipsychotic naïve/ free patients with first episode psychosis (FEP) and 30 healthy control (HCs) volunteers were included in the study. Participants were studied using functional magnetic resonance imaging whilst performing the N-Back working memory task. Blood oxygen level-dependent (BOLD) response, task performance, and the Positive and Negative Syndrome Scale (PANSS) were measured.

Results: My analysis revealed that patients (FEP) displayed blunted load-related (2-Back>1-Back) responses, relative to HCs, in the thalamus (p = 0.018) and hippocampus (p = 0.023). However, PANSS negative symptom scores did not correlate with this reduced BOLD response.

Discussion: My findings suggest that abnormal hippocampal and thalamic functioning could underlie the working memory impairments seen in unmedicated FEP but is unlikely to be related to the generation of negative symptoms.

2.2. Introduction

Schizophrenia and related psychotic disorders are severe mental illnesses characterized by positive, negative and cognitive symptoms [10]. Cognitive impairment between 0.5-1.5 standard deviations below performance in healthy matched control participants is apparent in patients from the first episode of illness across a number of cognitive tasks [6, 492-494], including those involving working memory [495]. Antipsychotic medications have been shown to improve positive symptoms [251], but are not effective for negative or cognitive symptoms [252] or have modest effects and are difficult to separate from other drug effects [253, 254]. Whilst several neural systems have been implicated the neural mechanisms underlying cognitive impairments and negative symptoms remain unclear [81, 136]. Understanding these mechanisms is important to help identify new treatment approaches [174].

Working memory (WM) is frequently impaired in schizophrenia and related psychotic disorders [443] (see section 1.3.3.7 for further details) and has been directly associated with the presence of negative symptoms [399] (see section 1.3.3.6), suggesting that a common neurobiological process could underlie both working memory impairment and negative symptoms. Working memory tasks engage a network of brain regions including the dorsolateral prefrontal cortex (DLPFC), hippocampus and thalamus [318, 347, 350, 351, 407, 458] (see section 1.6.3); key regions implicated in the pathophysiology of psychosis. During working memory tasks reduced activation has been reported in DLPFC [294], thalamus [294, 413, 496-498] and hippocampus [499] in first episode patients compared to healthy controls in some, but not all studies [457] (see 1.6.3.4). The inconsistency between studies may be due to the inclusion of medicated patients in some studies, as antipsychotic treatment has been found to affect the functional MRI signal [277, 280, 500] or that previous studies have not focused on working memory load, instead comparing

the working memory condition with an attentional control condition [294, 296, 457, 461, 476, 479, 481, 482, 501].

The predictions that can be made from this are that from these regions will have reduced activation, as compared to healthy controls. In view of this and the association between working memory impairment and negative symptoms, I aimed to investigate whether neural correlates during an increased working memory load would be reduced in these regions in drug free first episode patients, and secondly to determine whether this BOLD response was related to the severity of negative symptoms. I hypothesised that there would be less activation in the DLPFC, thalamus and hippocampus during high working memory load relative to lower load in patients than controls. I also hypothesised that lower activation in patients would be directly associated with greater severity of negative symptoms.

(Intentional Break)

2.3. Methods

2.3.1. Population and Sample

The East of England-Cambridge East NHS Research Ethics Committee approved this study. All participants provided informed written consent to participate.

2.3.1.1. Patient Group (n = 29)

45 patients were recruited into the study. Inclusion criteria were: a diagnosis of a psychotic disorder according to ICD 10 criteria [502, 503] which was diagnosed by their Early Intervention Team; presenting to the service with their first episode of psychosis [504]; total lifetime exposure to antipsychotic drugs of less than 4 weeks; and not currently taking an antipsychotic. Sixteen patients were excluded from the analysis for the following reasons: too much movement (FEP = 2); poor N-Back performance (<50% correct responses, FEP = 5); did not perform the N-Back task (FEP = 4); or were already taking antipsychotic medication (FEP = 5). Leaving a final group of 29 patients.

Potential participants were identified by the early intervention (EI) teams. After initial presentations to the EI teams, I would attend weekly multiple disciplinary meetings with the teams to enquire about new or existing patients who might be suitable for the study. These were patients with a diagnosis of first episode psychosis and who were either drug naïve, drug-free, or so-called refusers, who were patients who were currently refusing to start medication but may change their minds in the near future. Once these patients had been identified, their care coordinator or nurse would kindly ask them if they were interested in taking part in my study. If the patient was interested in taking part and was happy to provide me with their telephone number, I would arrange a time to meet them either in clinic face-to-face or telephone them to talk through the study and provide them with a patient information sheet.

2.3.1.2.   Healthy Control Group (n = 30)

33 healthy controls were recruited through advertisements in local newspapers and local participant recruitment service, MindSearch. Healthy controls were also excluded at the screening stage if they had a history of diagnosis and/or treatment for psychiatric or neurological disorders. No current or lifetime history of an Axis-I disorder as determined by the Structured Clinical Interview for DSM-IV-TR (SCID-I/P; [505] and no family history of an Axis-I disorder in first and second-degree relatives as examined by the Family Inventory for Genetics studies (FIGS; [506]. Three healthy controls were excluded from the analysis for the following reason: too much movement (HC = 3); Leaving a final group of 30 controls.

2.3.1.3.   Exclusion criteria for all participants:

Any contraindications to MRI scanning, pregnancy/breast feeding, a history of neurological or psychiatric disorder other than a psychotic disorder in the patient group; head trauma or neurodegenerative disorders, substance dependence (other than to tobacco) or any major medical condition that could compromise scanning safety (such as unstable severe asthma); excess movement during scanning (defined as a frame-wise displacement threshold of 0.5mm or greater [507]); or performance below 50% correct on 0-Back, 1-Back and 2-Back conditions to ensure performance was well above chance.

2.3.2.   Clinical Measures:

Four assessments were completed in total and in the following order: Positive and Negative Syndrome Scale (PANSS), National Adult Reading Test (NART), Global Assessment of Functioning (GAF) and a medication history. I will now describe the structure of each assessment. This first assessment was the Structured Clinical Interview for the Positive and Negative Syndrome Scale (SCI-PANSS) [508], which contains 179 questions and lasts between 30-40 minute. The question

sections refer to one of the thirty items on the PANSS scales (covering positive symptoms (items P1-7), negative symptoms (items N1-7), and general symptoms (items G1-16). The PANSS measures the severity of symptoms [509]. The Marder scale is not a separate scale but is derived from the PANSS scale. It takes 5 of the 7 negative symptoms from the PANSS (removing abstract and stereotyped thinking) to provide potentially a more valid measure of negative symptoms [510]. Premorbid IQ was assessed with the National Adult Reading Test (NART) [511] where participants were asked to read aloud a list of 50 words. 1 point was assigned for every correct pronunciation out of a possible total of 50 points/words. Patients' global function was measured using the Global Assessment of Functioning (GAF) [512]. The GAF ranges in score from 100 (superior functioning) through 1 (persistent danger of hurting self or others). 0 is scored on the GAF when there is insufficient information.

2.3.3. fMRI scanning:

2.3.3.1. Image acquisition:

Images were acquired on a 3T GE Signa scanner at the Centre for Neuroimaging Sciences, London, England. T2*-weighted images were acquired using a gradient echo sequence (repetition time of 2000ms and echo time of 30ms) with 39 slices of 3.5mm thickness (with 0.5mm gap) to produce 186 volumes (as well as 4 dummy acquisitions) across the N-Back task *[513]*. High-resolution whole brain T1-weighted structural images are acquired to facilitate mapping of the functional data into MNI space. The Field of View (FOV) was 24mm², with a 64*64 matrix, covering the whole brain with axial slices angled parallel to the AC/PC line. The total time for the fMRI N-Back sequence was 6 minutes and 20 seconds.

During the N-Back task, participants were asked to monitor the identity of a series of letters and to indicate when the currently presented stimulus was the same as the one presented *n* trials

previously; where *n* is 0, 1 or 2 by pressing the right-hand bottom of a two-button box. Where

n=0 they are instructed to respond when a given letter appeared and do not need to remember

the letters appearing. The three conditions were presented in 12 alternating 30-second blocks in

a pseudo-random order. Reaction time and response accuracy were recorded online.

Participants were given a practice session on a laptop with instructions that consisted of single

blocks of 12 of each condition before they went into the scanner. The order of difficulty increases

from 0-back (motor control condition) to 1-back to 2-back (most difficult).

2.3.3.2.   Image Analysis:

As my hypotheses concerned the neural basis of working memory load, I performed a 2x2 ANOVA

to look at load-by-group interaction and then used independent t-tests as my primary analysis

focused on the neural engagement during increased working memory load (i.e.: the 2-back

relative to the 1-back condition). Data were analysed using Statistical Parametric Mapping (SPM8;

Wellcome Department of Cognitive Neurology, London) running in MATLAB 8.0 (The MathWorks,

Natick, Massachusetts). During pre-processing, all volumes were realigned to the first volume of

the timeseries, a mean image was generated, in keeping with default 2 pass, approach the

volumes in the timeseries were then brought into alignment with the mean image. The affine

transformations required to bring the functional and structural data into alignment (i.e., co-

registration) were estimated using the mean image and then applied to the T2* timeseries.

Finally, the co-registered data was normalized into standard stereotactic space (template

provided by the Montreal Neurological Institute) using transformations derived from the unified

segmentation of the T1-weighted structural image. Finally, the normalised fMRI data was

smoothed with an 8mm full width half maximum (FWHM) smoothing kernel. Pre-processed data

quality was assured via visual inspection and in-house scripts.

It was a block design, so the onset time and duration of each block was isolated from the playlist. Additionally, participants reaction times on target trials were extracted.

The trial timing information was combined (convolved) with a model of the BOLD response, termed the canonical BOLD response. The resulted explanatory variables encoded the expected BOLD response associated with 8 conditions:

1.   1-Back blocks

2.   2-Back blocks

3.   0-Back blocks

4.   1-Back correct trials

5.   2-Back correct trials

6.   0-Back correct trials

7.   False Positives

8.   Misses

In addition to the standard 6 motion regressors, scan-nulling regressors were also added to the design matrix to model periods of large volume-to-volume motion (i.e., movement spikes) above a predefined threshold (0.5mm).

I used a script to calculate how much each participant moved from one volume to the next (frame-wise displacement) and flagged those time points where the movement exceeded my threshold of 0.5mm. I used a second script to created binary regressor columns to model each of the movements that exceeded the 0.5mm threshold as separate events. The third script that I used

combined these columns with the 6 motion parameters into one MATLAB file that was inputted into SPM for the 1st level model. Participants with more than 5 additional movement columns/spikes over the duration of the task were excluded from further analysis. Following parameter estimation contrast of parameters estimates were created, specifically for 2-Back>1-Back, 1-Back>0-Back and 2-Back>0-Back. I used the following script to formally test for head movement by calculating the average head movement per participant during the fMRI sequence:

```
root =
'/home/k1197916/Fiona_Share/N_back/1st_Level_Correct_Trials_Only/2nd_Level_Analysis/Basel
ine_2_Sample_t_test/min_treated_removed/con8'

cd(root)

load SPM.mat

move =[]

for i=1:length(SPM.xY.VY)

    cd('/home/k1197916/Fiona_Share/N_back/')

    [direct,file,ext] = fileparts(SPM.xY.VY(i).fname)

    pathparts = strsplit(direct,filesep)

    folder = dir([pathparts{end}(2:end) '*'])

        for j = 1:length(folder)

         cd('/home/k1197916/Fiona_Share/N_back/')

        display(folder(j).name)

        cd(folder(j).name)

        file= dir('*rp*.txt')
```

```
    for k =1:length(file)

        pwd

        rp =load(file(k).name);

        u = rp(2:end,1:3);

        v = rp(1:end-1,1:3);

        delt = u - v;

        d = (sum(delt .^ 2,2)) .^.5;

        total_move = sum(d);

        move = [move; total_move]

    end

  end

end
```

Parameter estimation with autocorrelation modelled using a first order autoregressive function (AR(1)). Following parameter estimation, these contrasts of parameter estimates were generated:

1. 1-Back>0-Back

2. 1-Back<0-Back

3. 2-Back>0-Back

4. 2-Back<0-Back

5. 1-Back>2-Back

6.   1-Back<2-Back

7.   1-Back+2-Back>0-Back

8.   1-Back+2-back<0-Back

Subject-specific contrasts, generated in the fixed effects models, were taken forward to group-level random effects analysis. Specifically, the following models were employed:

1.   2x2 ANOVA to look at load-by-group interaction with 1-Back>0-Back and 2-Back>0-Back as the levels

2.   Independent t-tests as my primary analysis focused on the neural engagement during increased working memory load

One sample t-tests were used to look at the main effect of the task at baseline in patients and controls. A two-sample t-test was used to compare the healthy control group with the patients with first-episode psychosis at baseline. The whole brain analyses were exploratory. I employed a region-of-interest analysis approach. A priori ROI masks were generated for the hippocampus and thalamus bilaterally using the Automated Anatomical Labelling (AAL) Atlas in WFU Pick-Atlas [514]. The DLPFC ROI was also created in WFU Pick-Atlas *[514]* using TD Brodmann Areas + (comprised of BA9 and BA46 [318, 515, 516]). I used cluster-based inference for the exploratory whole brain analysis. For the hypothesis-led ROI analyses, statistical maps were initially thresholded at an uncorrected height threshold of $p<0.001$, I applied SVC with family wise error on the basis of peak amplitude (SVC; pFWE<0.05) using each ROI.

In all cases, unless otherwise stated, a result was deemed significant if it survived family-wise error (FWE) correction based on cluster extent (pFWE corrected <0.05) within the whole brain or for the peak (s.v.c pFWE corrected <0.05) in predefined small volumes (i.e., ROI masks) using the SPM default uncorrected height threshold of p<0.001. Cluster-based inference does use an uncorrected cluster-forming threshold of p<0.001. I identified the minimal cluster extent (k) that was associated with a significant (p<0.05) clusterwise FWE. I then re-thresholded the map with the smallest k-value for a significant cluster-wise FWE corrected cluster now used as the minimum cluster extent to remain on the map. I applied no peak level correction as the minimal extent threshold and left the height threshold at p<0.001 uncorrected. The whole brain analyses were exploratory as they were not specified *a priori*.

The hypotheses specified *a priori* three ROIs; the DLPFC, hippocampus and thalamus. Due to the high dimensionality of the data, fMRI comes with the risk of type I errors because of the large quantity of concurrent statistical tests. The severity of the problem is described by the smoothness of the data and the number of voxels tested. In the literature, there are two main approaches to multiple testing which are cluster or voxel-wise control of family-wise error (FWE), and voxel-wise control of the false discovery rate (FDR). In this thesis I used FWE for ROIs and then applied the Bonferroni correction to reduce p-value further control the type I error rate by adjusting for the number of primary ROIs. Thus, hypothesised results were considered significant if the family-wise error corrected p<0.0167.

To investigate if differences in age, gender and 2-back performance could contribute to findings, I added in additional exploratory post-hoc analyses to investigate these as 3 separate covariates.

2.3.3.3. Integration of fMRI, and PANSS data:

For contrasts where small-volume correction identified significant between-group differences, I extracted mean β-weights for each patient over the ROI (DLPFC (equivalent to Brodmann areas 9 and 46), hippocampus and thalamus) during the N-Back working memory task (2-Back>1-Back contrast) using MarsBar in SPM8 and correlated these with the PANSS or Marder negative symptom scores for patients in SPSS.

2.3.4. Statistical analysis

The effect of group on demographics and clinical measures scores were tested using analysis of variance for parametric variables and the Mann-Whitney U tests for non-parametric variables after checking for the equality of variance using the Levene test, or chi-squared/Kruskal-Wallis for categorical data. A mixed factorial 3x2 ANOVA was used to analyse the N-Back behavioural scores where the three levels of the N-Back task (2-back, 1-back, and 0-back) were the within-subject factors, and group (patient or healthy control) was the between-subjects factor. Where the Mauchley's test indicated that the assumption of sphericity had been violated, the degrees of freedom were corrected using Huynh-Feldt estimates of sphericity ($\varepsilon < 0.75$).

To investigate the relationship between negative symptoms with BOLD response, Pearson's correlations were used ($p < 0.05$ two-tailed). β values were extracted using MarsBar 0.44 SPM toolbox (http://marsbar.sourceforge.net/) separately for the left hippocampus, right hippocampus, left thalamus and right thalamus, and were plotted against PANSS negative sub-scores or Marder negative scores.

2.4. Results
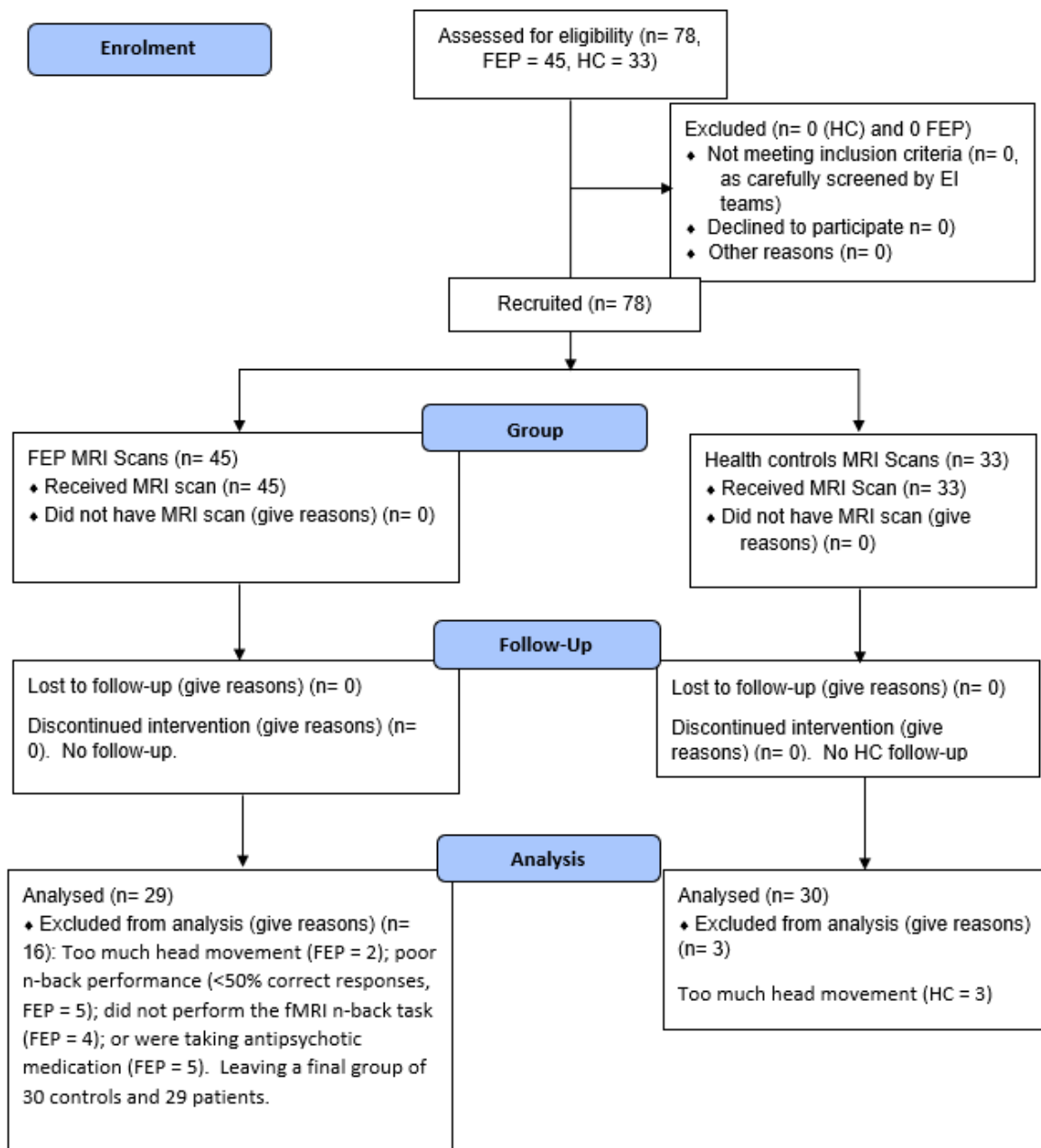
Figure 10 CONSORT 2010 Flow Diagram

2.4.1. Clinical and demographic characteristics of the sample (see Table 3):

There were no significant differences between the groups in terms of age (t(55) = 0.813, p = 0.422), gender (χ^2 (1) = 0.763, p = 0.383), or premorbid IQ (NART) t(29) = 1.575, p = 0.126). As expected, the GAF was significantly lower in the patient group (t(47) = 15.295, p < 0.05). Age and gender were balanced between the groups therefore this factor was not controlled for in the imaging analysis.

Table 3: Group Demographics and clinical characteristics *GAF=Global Assessment of Functioning; NART - National Adult Reading Test; FES – First Episode Psychosis; BP – Bipolar; PD – Psychotic Depression; PANSS - Positive and Negative Syndrome Scale*

Variable	Patients, n=29	Healthy controls, n=30
Sex (female:male)	5:24	8:22
Age ((mean years) [SD])	25.15 [4.75]	24.68 [3.88]
Duration of Untreated Illness (months [sd])	23.33 [18.35]	N/A
GAF	49.67 [13.65]	96.45 [4.80]
NART	31.93 [7.65]	36.38 [8.02]
Diagnosis FES/BP/PD	17/10/2	N/A
PANSS Positive	18.83 [6.8]	N/A
PANSS Negative	15.26 [6.6]	N/A
Marder Negative	15.76 [7.9]	N/A
PANSS General	36.70 [9.9]	N/A
PANSS Total	70.80 [19.8]	N/A
Medication status	13/29 Drug-Naïve; 16/29 Drug-free	N/A

2.4.2. N-Back Behavioural Data:

The results showed that there was a main effect of the response accuracy of the N-Back task $F(1.757, 110.144) = 10.87$, $p < 0.001$. There was a significant N-Back x Group interaction $F(2,20) = 3.17$, $p = 0.046$ whereby the ratings of patients and controls differed significantly on the N-Back behavioural scores. There was a significant main effect of group, $F(1,57) = 4.61$, $p = 0.036$. Bonferroni corrected pairwise comparison *post hoc* tests showed that the accuracy of the N-Back behavioural scores were not significantly different for 0-back or 1-back or 2-back ($p = 0.355$, $p = 0.118$ and $p = 0.021$ respectively - see Figure 11 and Table 4). All pairs must be compared to ascertain if the means are significantly different. To determine the number of pairwise differences needed to calculate the correct Bonferroni's probability value, the following calculation was use: there are $k = (a)(a-1)/2$ possible pairs where $a =$ the number of levels of the N-Back task (i.e., $a = 3$). $3(3-1)/2 = 3$ pairwise differences to consider and $p = 0.05/3 = 0.0167$. N-Back behavioural scores were the number of correct responses participants obtained out of 12 for each level of the N-Back task. Table 4 shows the means for each group at each level of difficulty of the task (i.e., 0-back, 1-back, and 2-back).
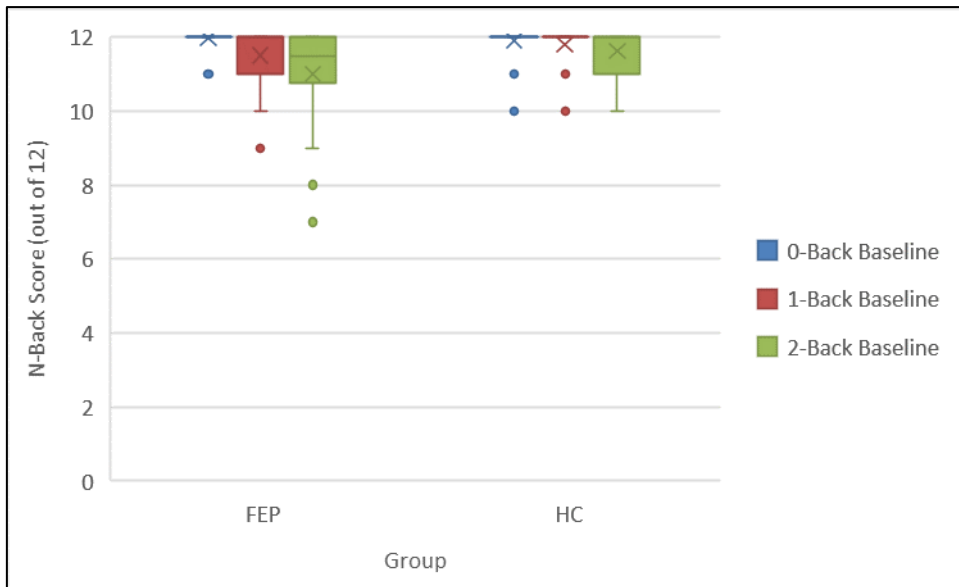
Figure 11: Boxplots of the mean N-Back behavioural Scores for patients with first episode psychosis (FEP) and healthy controls (HCs)

Table 4: N-Back Behavioural Data – number of correct responses (Mean [SD]) and Bonferroni-corrected post-hoc independent t-tests.

	First Episode Patients	Healthy Controls	Bonferroni-corrected post-hoc independent t-tests (p<0.0167)
0-Back	11.97 [0.19]	11.90 [0.40]	t(57) = -0.932, p = 0.355
1-Back	11.55 [0.88]	11.80 [0.48]	t(57) = 1.587, p = 0.118
2-Back	11.06 [1.22]	11.63 [0.61]	t(57) = 2.363, p = 0.021

2.4.3. fMRI results:

2.4.3.1. Formal Test of Head Movement

The results show that there was a significant effect of head movement between the groups $t(55)$ = 2.185, p = 0.021 (see Figure 12).
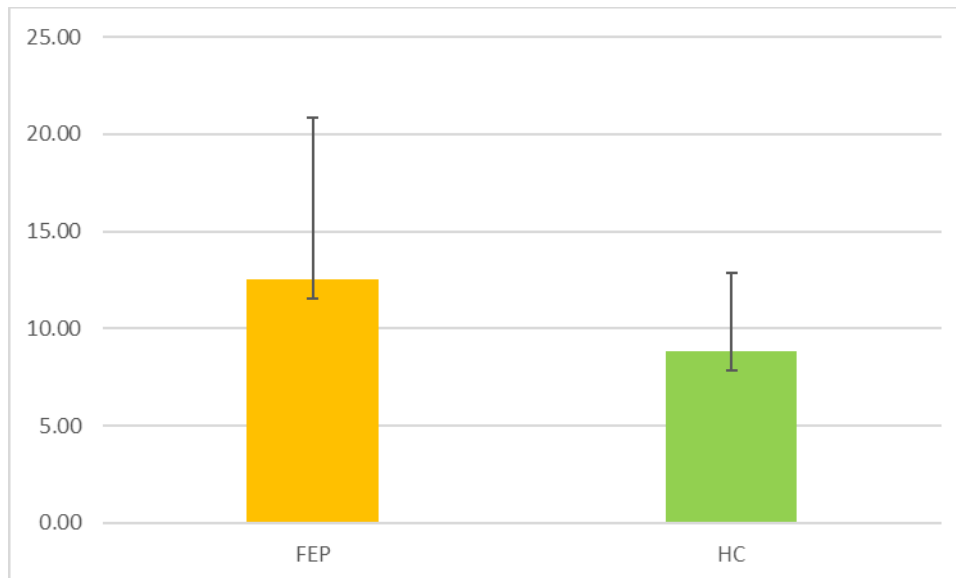
Figure 12: Mean Head Movement for Patients and Healthy Controls (in millimetres)

2.4.3.2. Main Effect of Task (in healthy controls):

The ROI analysis showed that there was significantly increased activation during 2-back>1-back BOLD response in the following areas: hippocampus, DLPFC and thalamus, (see Table 5, Figure 13, Figure 14, and Figure 15). I used FWE for ROIs and then applied the Bonferroni correction to reduce p-value further control the type I error rate by adjusting for the number of primary ROIs. Although they survived familywise error correction (pFWE<0.05), 8 out of 15 DLPFC ROI analyses and 2 out of the 3 thalamus ROIs did not reach the more conservative threshold based on the Bonferroni correction for the number of independent ROIs used (i.e., pFWE<0.0167). The hippocampus ROI did reach this conversative threshold - see Table 5.

Table 5: Region of Interest (ROI) Increased Activation during the N-Back Task in Controls for 2-Back>1-Back Contrast

ROI	Region	L/R	MNI Coordinates			No. of voxels	z-score	Peak Level (pFWE corrected)	FWE error corrected with additional Bonferroni Correction (p<0.0167)
			x	y	z				
Hippocampus	Hippocampus	R	22	-16	10	17	3.88	0.009	Significant
DLPFC	Middle Frontal Gyrus	R	36	30	34	350	5.37	p<0.001	Significant
		R	32	36	30		5.02	p<0.001	Significant
		R	38	38	36		4.68	0.001	Significant
	Precentral Gyrus	L	-58	6	32	203	5.27	p<0.001	Significant
	Precentral Gyrus	L	-48	4	28		5.02	p<0.001	Significant
	Middle Frontal Gyrus	R	34	42	30	23	4.82	0.001	Significant
	Inferior Frontal Gyrus, Opercular Part	L	-36	4	28	3	4.57	0.002	Significant

			x	y	z	Cluster	T	p	
DLPFC	Inferior Frontal Gyrus, Triangular Part	R	44	38	24	46	4.55	0.002	Significant
	Middle Frontal Gyrus	R	38	36	16		3.81	0.037	N/S
	Middle Cingulate & Paracingulate Gyri	R	10	26	36	27	3.98	0.020	N/S
	Middle Frontal Gyrus	R	42	50	20	2	3.95	0.023	N/S
	Middle Frontal Gyrus	L	-42	24	34	106	3.86	0.031	N/S
		L	-36	30	34		3.79	0.040	N/S
	Inferior Frontal Gyrus, Triangular Part	L	-44	30	24	14	3.75	0.045	N/S
	Superior Frontal Gyrus, Dorsolateral	L	-10	26	36	20	3.74	0.048	N/S
Thalamus	Thalamus	L	-14	-6	0	43	4.02	0.005	Significant
		R	14	-8	16	37	3.58	0.019	Significant
		R	16	-12	18		3.49	0.041	N/S

*N/S = Not significant
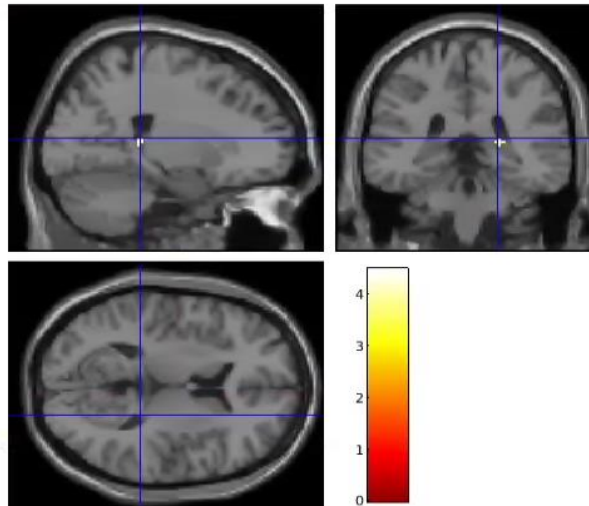
Figure 13: Statistical maps illustrating regions of significantly increased activation during the N-Back Task in Controls for the 2-Back>1-Back Contrast. For hypothesis-led ROI analyses, statistical maps were initially thresholded at an uncorrected height threshold of p<0.001, followed is family-wise error correction via small volume correction (SVC; pFWE<0.05) using hippocampus ROI. The peak is shown by the cross hair. The colour bar indicates the t value.
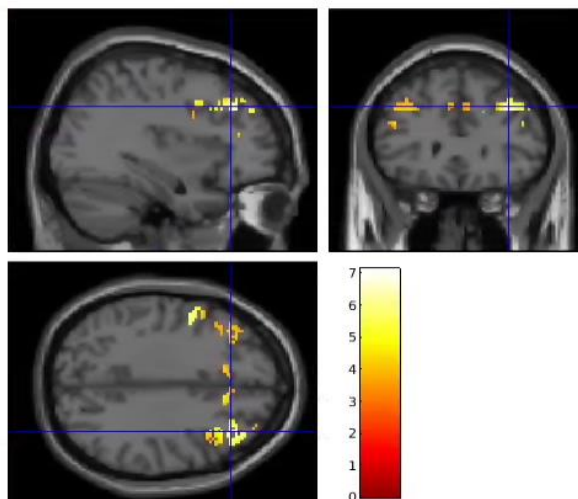
Figure 14: Statistical maps illustrating regions of significantly increased activation during the N-Back Task in Controls for the 2-Back>1-Back Contrast. For hypothesis-led ROI analyses, statistical maps were initially thresholded at an uncorrected height threshold of p<0.001,

followed is family-wise error correction via small volume correction (SVC; pFWE<0.05) using

DLPFC ROI. The peak is shown by the cross hair. The colour bar indicates the t value.
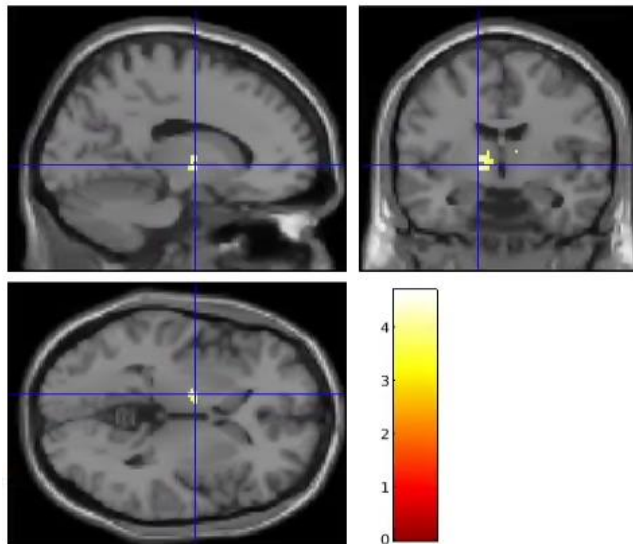
Figure 15: Statistical maps illustrating regions of significantly increased activation during the N-Back Task in Controls for the 2-Back>1-Back Contrast. For hypothesis-led ROI analyses, statistical maps were initially thresholded at an uncorrected height threshold of p<0.001, followed is family-wise error correction via small volume correction (SVC; pFWE<0.05) using thalamus ROI. The peak is shown by the cross hair. The colour bar indicates the t value.

Figure 16, Figure 17, and Figure 18 display images of the significant patterns of activation

associated with working memory at each level of difficulty. BOLD is a relative measure thus the

two conditions were contrasted for each figure. 2-Back>0-Back (see Figure 16) shows patterns of

activation when 2-back was contrasted with 0-back (pFWE corrected<0.05) in healthy controls.

The 2-back>0-Back contrast is shown in Figure 17 with p = 0.001 uncorrected, for illustrative

purposes. In Figure 18, I show the pattern of activation for 1-back compared to 0-back in healthy

controls. It did not survive family wise error (FWE) correction at (pFWE corrected<0.05) but is

shown at p = 0.001 uncorrected in Figure 18 for illustrative purposes.
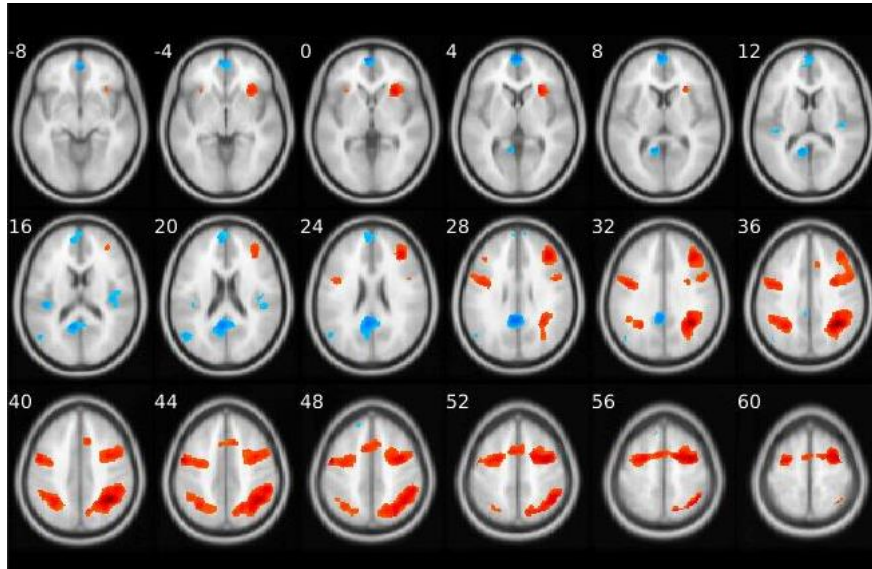
Figure 16: Patterns of significant activation associated with working memory comparing the hardest level of the task with the control condition (2-Back>0-Back) in healthy control participants (pFWE corrected<0.05), where the red indicates increases in activation (2-Back>0-Back) and blue indicates de-activation (0-Back>2-Back).
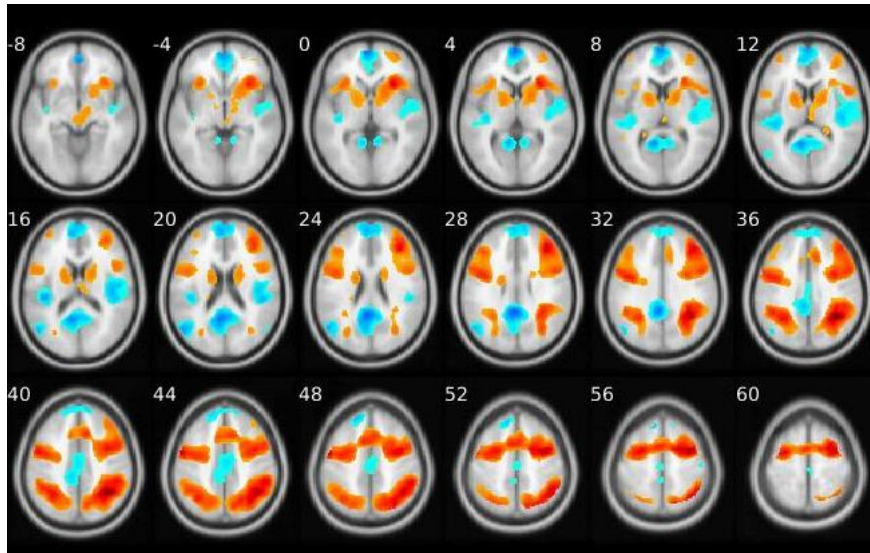
Figure 17: Patterns of significant activation associated with working memory comparing the hardest level of the task with the control condition (2-Back>0-Back) in healthy control participants. Results are shown using a statistical threshold of p=0.001 uncorrected for illustrative purposes. Red/orange indicates increases in activation (2-Back>0-Back) and blue indicates de-activation (0-Back>2-Back).
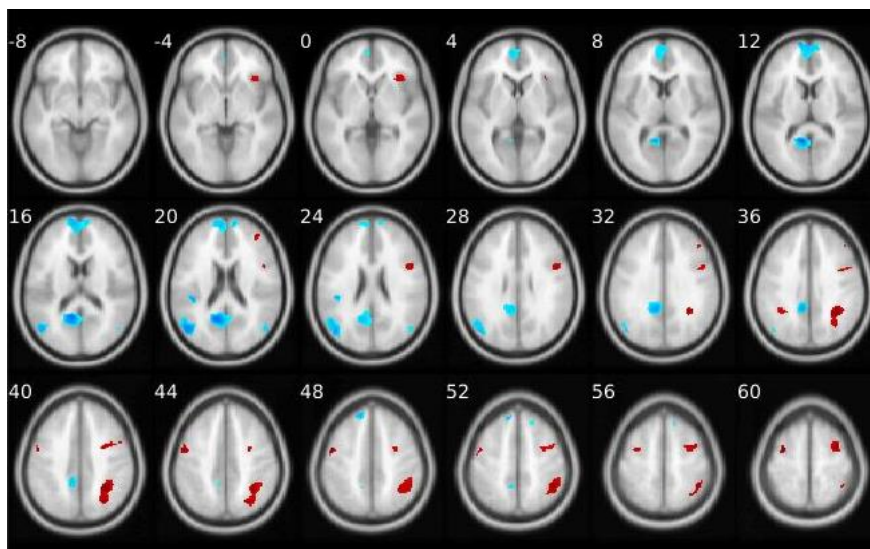
Figure 18: Patterns of activation associated with working memory comparing the easiest level of the task with the control condition (1-Back>0-Back) in healthy control participants p=0.001

uncorrected (for illustrative purposes). Red indicates increases in activation (1-Back>0-Back)

and blue indicates de-activation (0-Back>1-Back)

2.4.3.3. Main Effect of Task (in patients):

The ROI analysis showed that there was significantly increased activation during 2-back>1-back

BOLD response in: DLPFC and thalamus (see Table 6, Figure 19, and Figure 20). I used FWE for

ROIs and then applied the Bonferroni correction to reduce p-value further control the type I error

rate by adjusting for the number of primary ROIs. There was no significant activation in the

hippocampus ROI. Even though they survived familywise error correction (pFWE<0.05), all but

two ROIs survived Bonferroni correction (p = 0.020 and p = 0.038), both of which were in the

DLPFC.

(Intentional Break)

Table 6: Region of Interest (ROI) Increased Activation during the N-Back Task in Patients for 2-Back>1-Back Contrast

ROI	Region	L/R	MNI Coordinates x	y	z	No. of voxels	z-score	Peak Level (pFWE corrected)	FWE error corrected with additional Bonferroni Correction (p<0.0167)
DLPFC	Inferior Frontal Gyrus	L	-42	2	32	61	4.30	p<0.001	Significant
	Middle Frontal Gyrus	L	-42	4	38		3.99	p<0.001	Significant
	Middle Frontal Gyrus	R	42	30	38	88	4.18	0.013	Significant
	Middle Frontal Gyrus	-34	44	30	34	23	4.06	0.020	N/S
	Medial Frontal Gyrus	R	10	26	36	17	3.88	0.038	N/S
Thalamus	Thalamus	L	-18	-10	4	12	3.55	p<0.001	Significant
		L	-16	-8	0		3.49	p<0.001	Significant

N/S = not significant
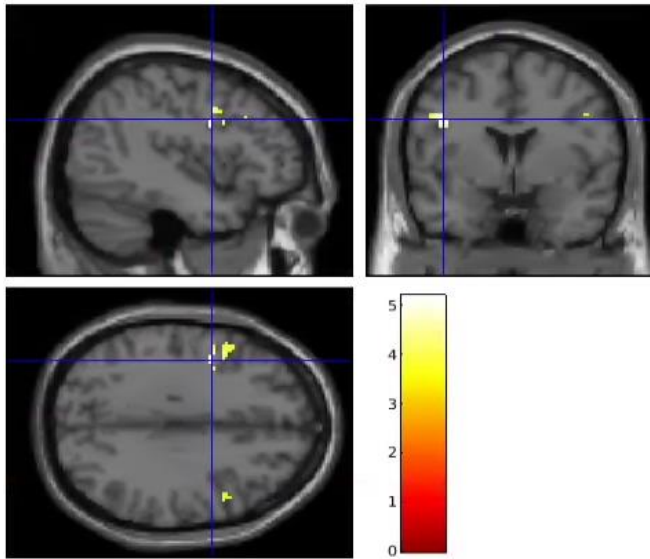
Figure 19: Statistical maps illustrating regions of significantly increased activation during the N-Back Task in Patients for the 2-Back>1-Back Contrast. For hypothesis-led ROI analyses, statistical maps were initially thresholded at an uncorrected height threshold of p<0.001, followed is family-wise error correction via small volume correction (SVC; pFWE<0.05) using DLPFC ROI. The peak is shown by the cross hair. The colour bar indicates the t value.
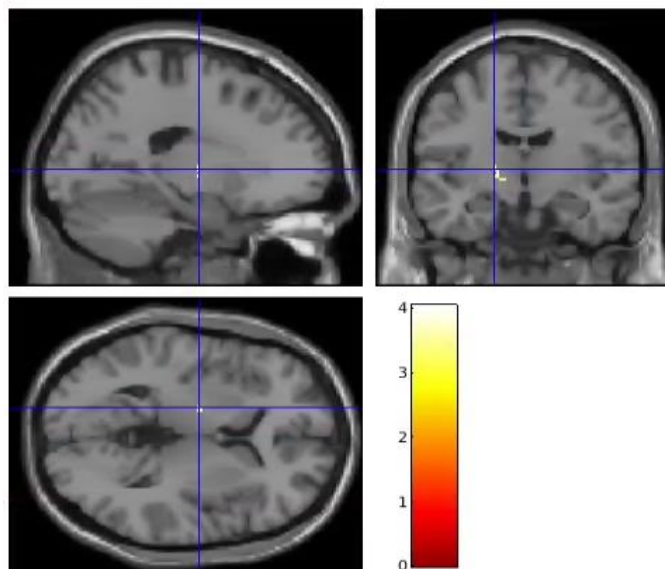
Figure 20: Statistical maps illustrating regions of significantly increased activation during the N-Back Task in Patients for the 2-Back>1-Back Contrast. For hypothesis-led ROI analyses,

statistical maps were initially thresholded at an uncorrected height threshold of p<0.001, followed is family-wise error correction via small volume correction (SVC; pFWE<0.05) using thalamus ROI. The peak is shown by the cross hair. The colour bar indicates the t value.

2.4.3.4.   First Episode Psychosis Patients vs Healthy Controls:

In whole-brain analyses (uncorrected height threshold of p<0.001) of the two (group) by two (WM load) interaction, there were no suprathreshold clusters for either contrast (((1-Back>0-Back) > (2-Back>0-Back)) or ((1-Back>0-Back) < (2-Back>0-Back))). There was no significant activation difference between patients and healthy controls during the exploratory whole brain analysis for 2-Back<1-Back contrast. There was significantly greater activation in healthy controls (HC) compared to patients (FEP) when participants performed the 2-back task compared to the 1-back condition in the right thalamus (p = 0.010) and right hippocampus (p = 0.007) (see Table 7, Figure 21, Figure 22, Figure 23 and Figure 24). There was no significant difference in activation between groups in the DLPFC (p = 0. 098) (See Table 7).

Table 7: Region of Interest Analysis- Activation is greater in healthy controls than FEP patients during increased working memory load (SVC pFWE<0.05 at display threshold of p<0.001 unc)

ROI	L /R	MNI Coordinates			No. of voxels	z-scores	P- value at peak (pFWE corrected)	FWE error corrected with additional Bonferroni Correction (p<0.0167)
		x	y	z				
Thalamus	R	20	-30	12	22	3.79	0.010	Significant
Hippocampus	R	22	-34	10	15	3.94	0.007	Significant
DLFPC	L	-56	4	30	13	3.53	0.098	Not Significant
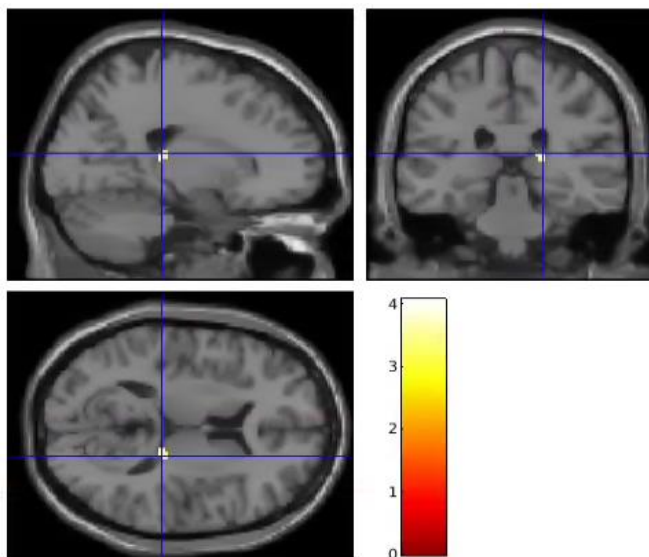
Figure [21]: This shows regions of significantly greater activation in healthy control participants relative to patients for 2-back>1-back contrast at baseline in the thalamus. For hypothesis-led

ROI analyses, statistical maps were initially thresholded at an uncorrected height threshold of

p<0.001, followed is family-wise error correction via small volume correction (SVC; pFWE<0.05)

using thalamus ROI. The peak is shown by the crosshair. The colour bar indicates the t value.
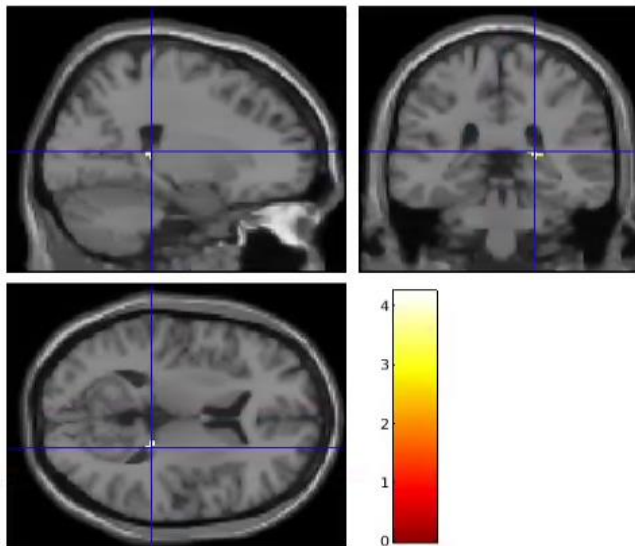
Figure 22: This shows regions of significantly greater activation in healthy control participants relative to patients for the load 2-back>1-back contrast at baseline in the hippocampus. For hypothesis-led ROI analyses, statistical maps were initially thresholded at an uncorrected height threshold of p<0.001, followed is family-wise error correction via small volume correction (SVC; pFWE<0.05) using hippocampus ROI. The peak is shown by the crosshair. The colour bar indicates the t value.
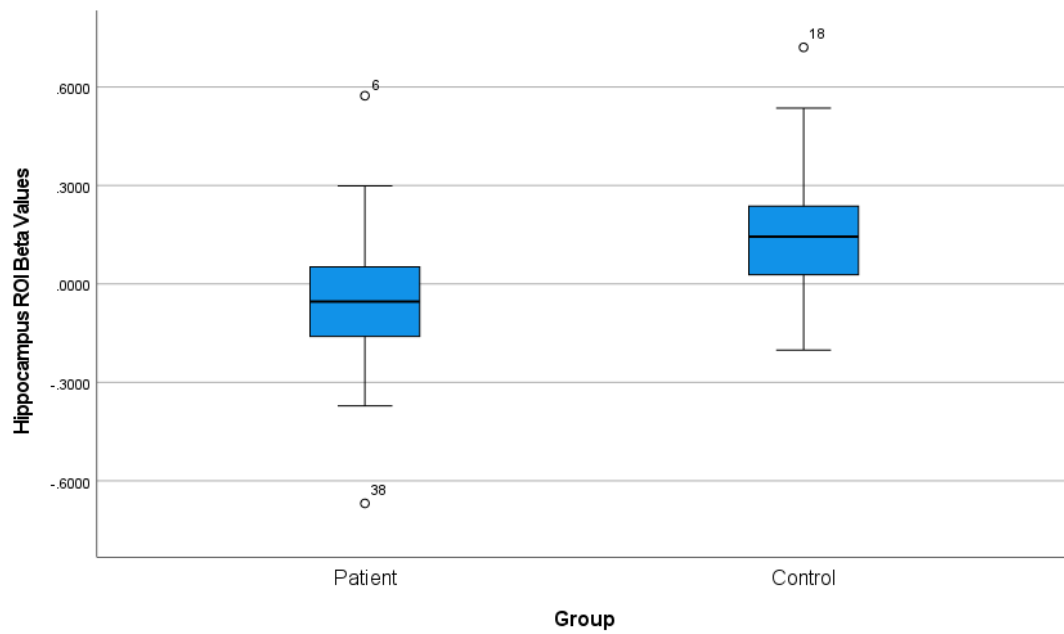
Figure 23: Boxplot of beta values for patients with first episode psychosis and healthy controls, in the hippocampus ROI.
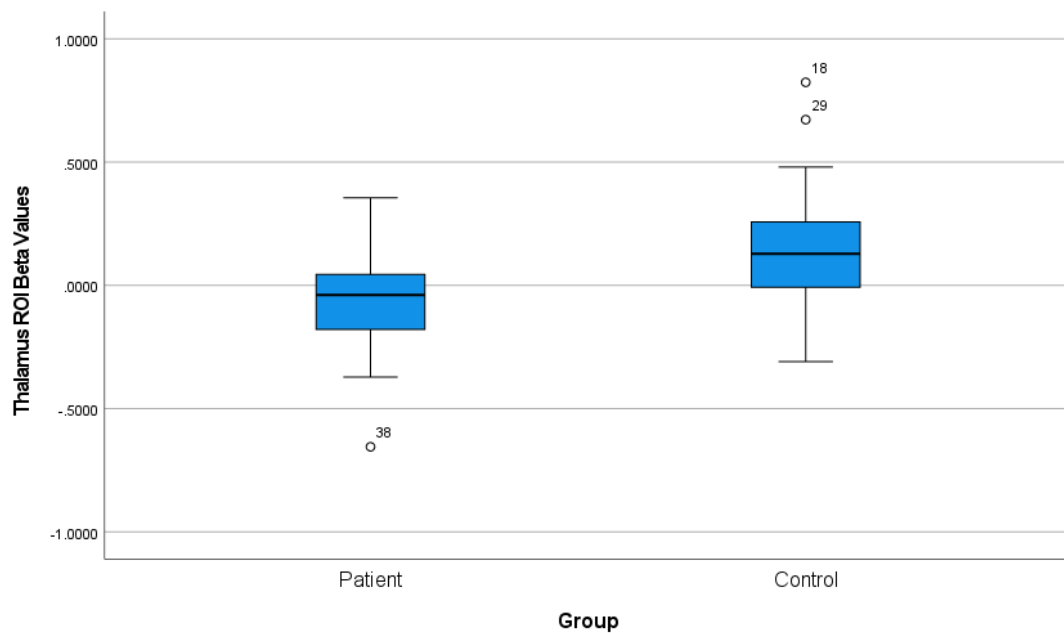
Figure 24: Boxplot of beta values for patients with first episode psychosis and healthy controls, in the thalamus ROI.

2.4.3.5. Nuisance Covariates - Age, Gender, and 2-Back Performance

After controlling for age, gender, and task-performance, there was significantly greater activation in healthy controls (HC) compared to patients (FEP) when participants performed the 2-back task compared to the 1-back condition in the right thalamus (p = 0.018) and right hippocampus (p = 0.023) (see Table 8, Figure 25 and Figure 26). Although they survived familywise error correction (pFWE<0.05), they did not reach the more conservative threshold based on the Bonferroni correction for the number of independent ROIs used (i.e., pFWE<0.0167). There was no significant difference in activation between groups in the DLPFC (p = 0.063).

Table 8: Region of Interest Analysis- Activation is greater in healthy controls than FEP patients during increased working memory load (SVC pFWE<0.05 at display threshold of p<0.001 unc) where nuisance regressors encoding age, gender and 2-back performance were included.

ROI	L/R	MNI Coordinates			No. of voxels	z-scores	Peak Level (pFWE corrected)	FWE error corrected with additional Bonferroni Correction (p<0.0167)
		x	y	z				
Thalamus	R	20	-30	12	3	3.44	0.030	N/S
Hippocampus	R	22	-34	10	5	3.52	0.030	N/S

*N/S not significant
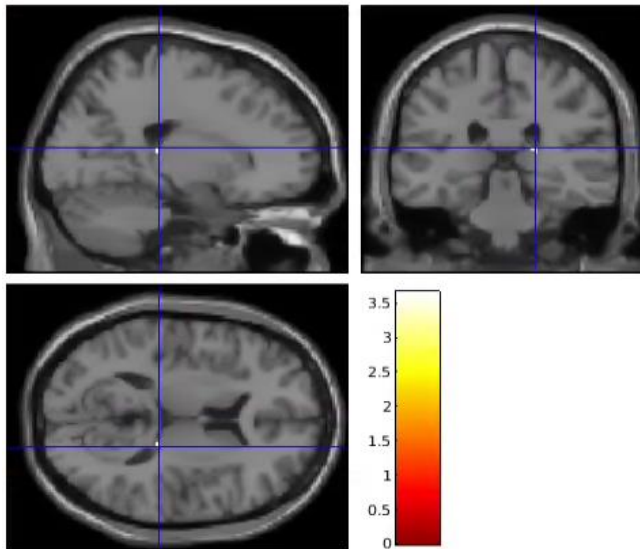
Figure 25: This shows regions of significantly greater activation in healthy control participants relative to patients for the load 2-back>1-back contrast at baseline in the thalamus. For hypothesis-led ROI analyses, statistical maps were initially thresholded at an uncorrected height threshold of p<0.001, followed is family-wise error correction via small volume correction (SVC; pFWE<0.05) using thalamus ROI, where nuisance regressors encoding age, gender and 2-back performance were included. The peak is shown by the cross hair. The colour bar indicates the t value.
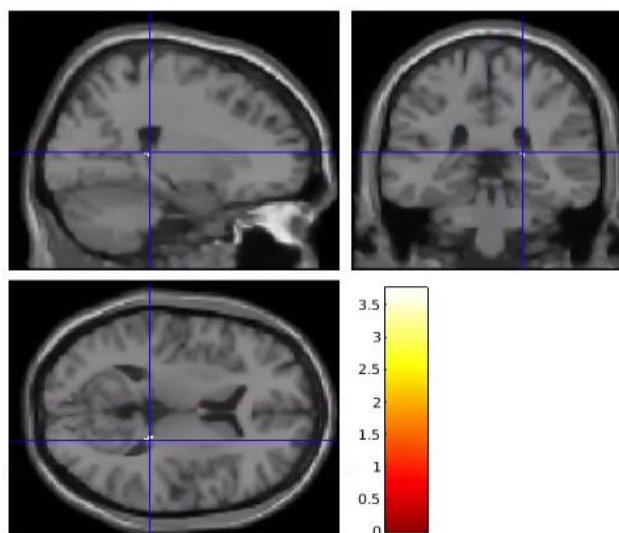
Figure 26: This shows regions of significantly greater activation in healthy control participants relative to patients for the load 2-back>1-back contrast at baseline in the hippocampus. For

hypothesis-led ROI analyses, statistical maps were initially thresholded at an uncorrected height threshold of p<0.001, followed is family-wise error correction via small volume correction (SVC; pFWE<0.05) using hippocampus ROI, where nuisance regressors encoding age, gender and 2-back performance were included. The peak is shown by the cross hair. The colour bar indicates the t value.

2.4.4. Relationship between negative symptoms and BOLD activation during working memory performance:

For ROIs where there was evidence of significant group differences in activation (i.e., small volume correction), I examined the relationship between mean neural activation in this region and negative symptom severity. There were no significant correlations with PANSS negative sub-scale scores or Marder negative scores and BOLD response in any of the regions of interest (see Table 9, Figure 27, Figure 28, Figure 29, Figure 30, Figure 31, Figure 32, Figure 33, and Figure 34).

Table 9: No significant correlations between negative symptoms and BOLD response in the thalamus and hippocampus.

	PANSS Negative Sub-Scale	Marder Negative Scores
Left Thalamus	r = 0.134, p = 0.504	r = 0.147, p = 0.465
Right Thalamus	r = 0.141, p = 0.483	r = 0.146, p = 0.468
Left Hippocampus	r = -0.193, p = 0.317	r = -0.159, p = 0.409
Right Hippocampus	r = -0.184, p = 0.340	r = -0.201, p = 0.297
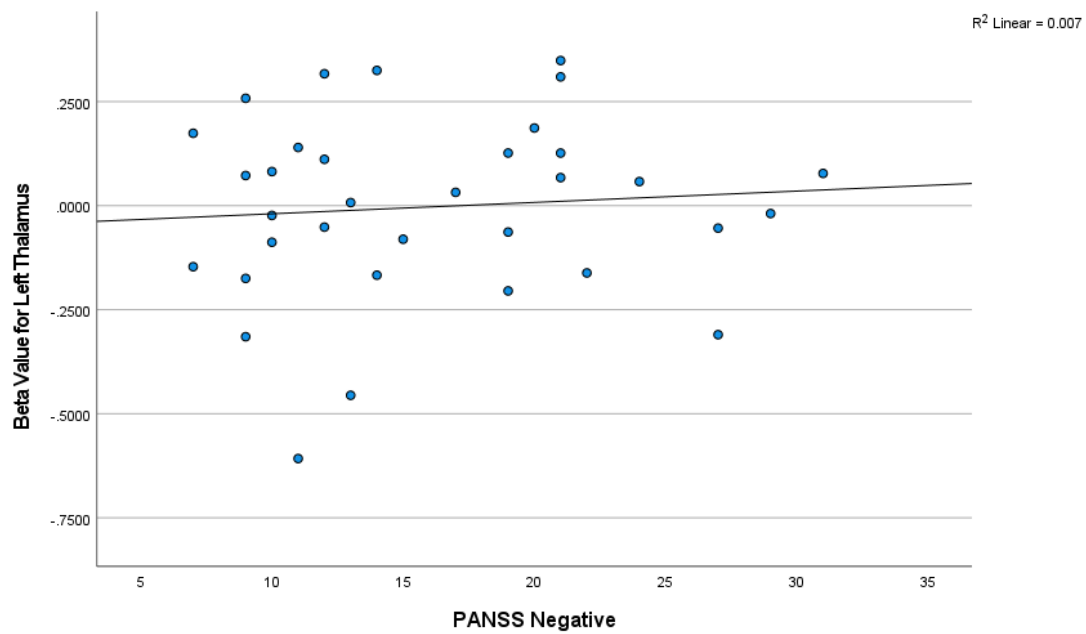
Figure 27: Correlations with Negative Symptoms and Beta Values from Left Thalamus
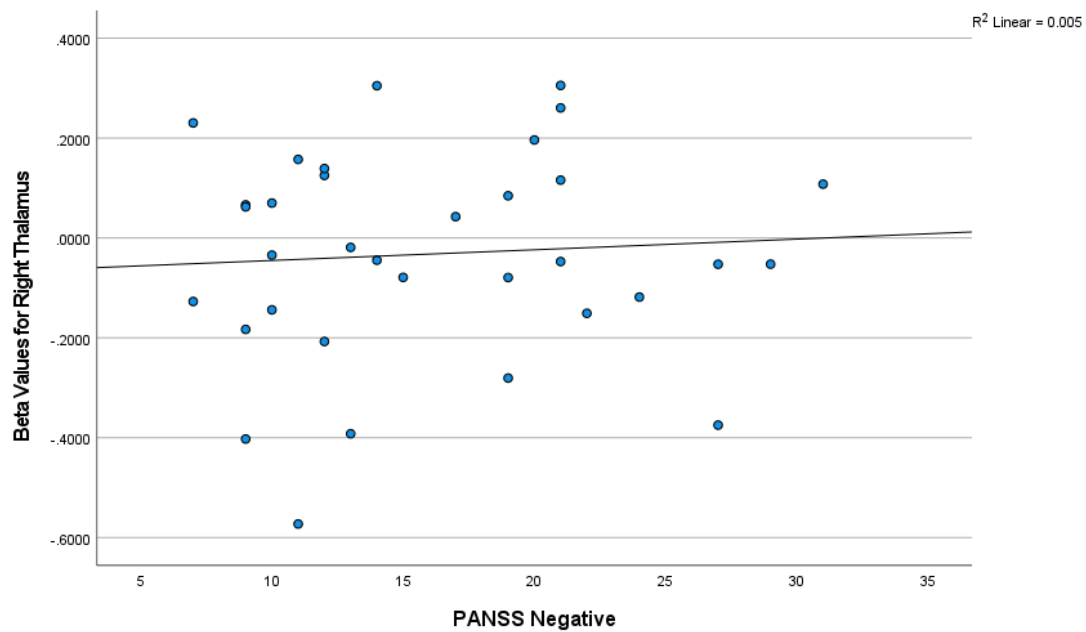
Figure 28: Correlations with Negative Symptoms and Beta Values from Right Thalamus
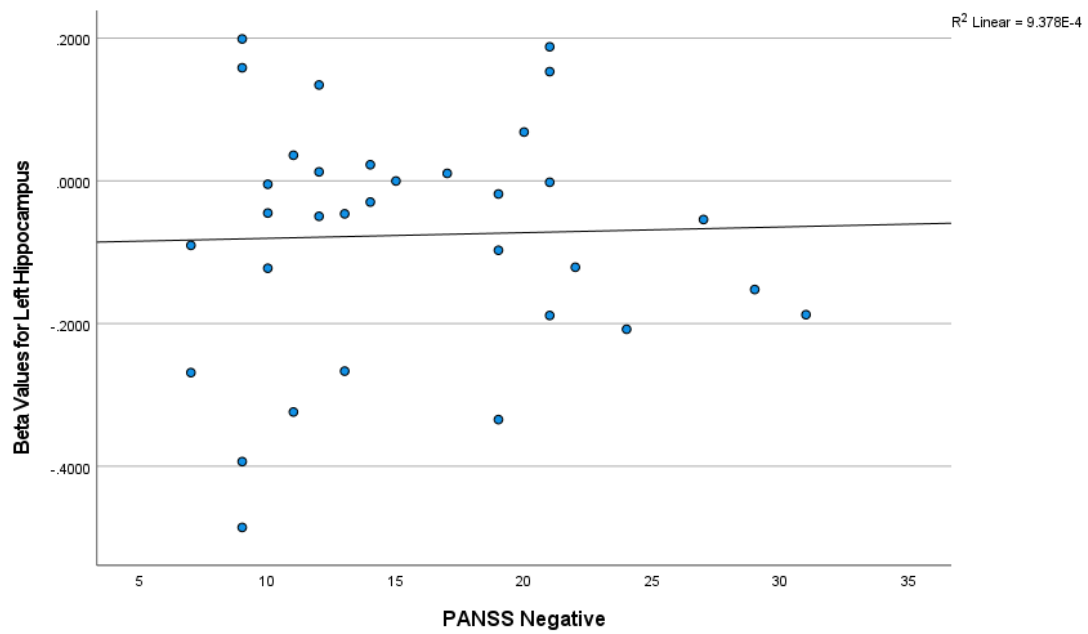
Figure 29: Correlations with Negative Symptoms and Beta Values from Left Hippocampus
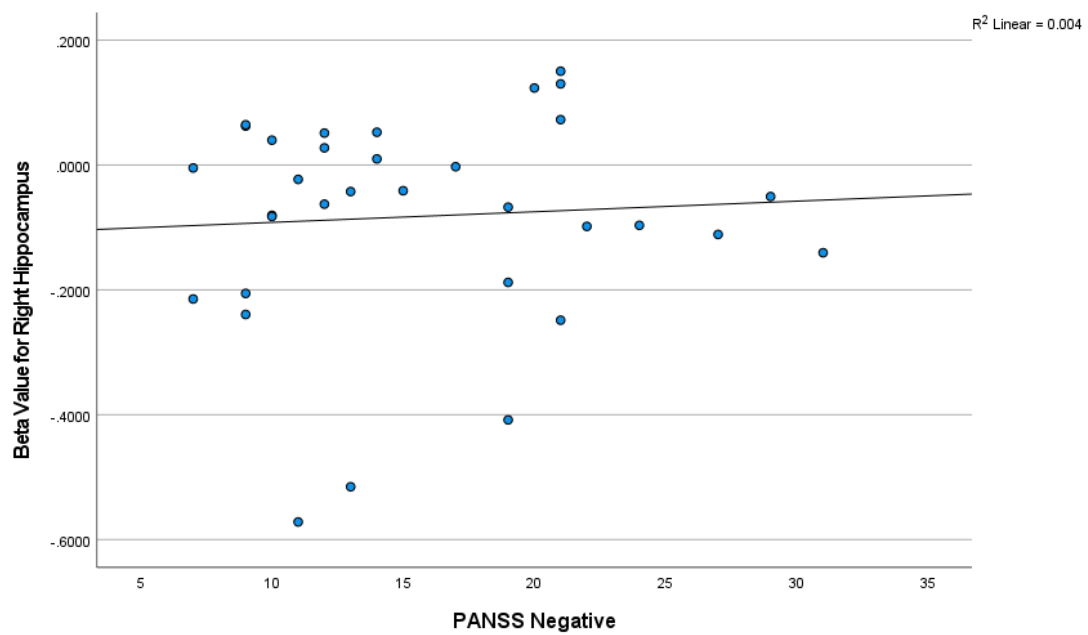
Figure 30: Correlations with Negative Symptoms and Beta Values from Right Hippocampus
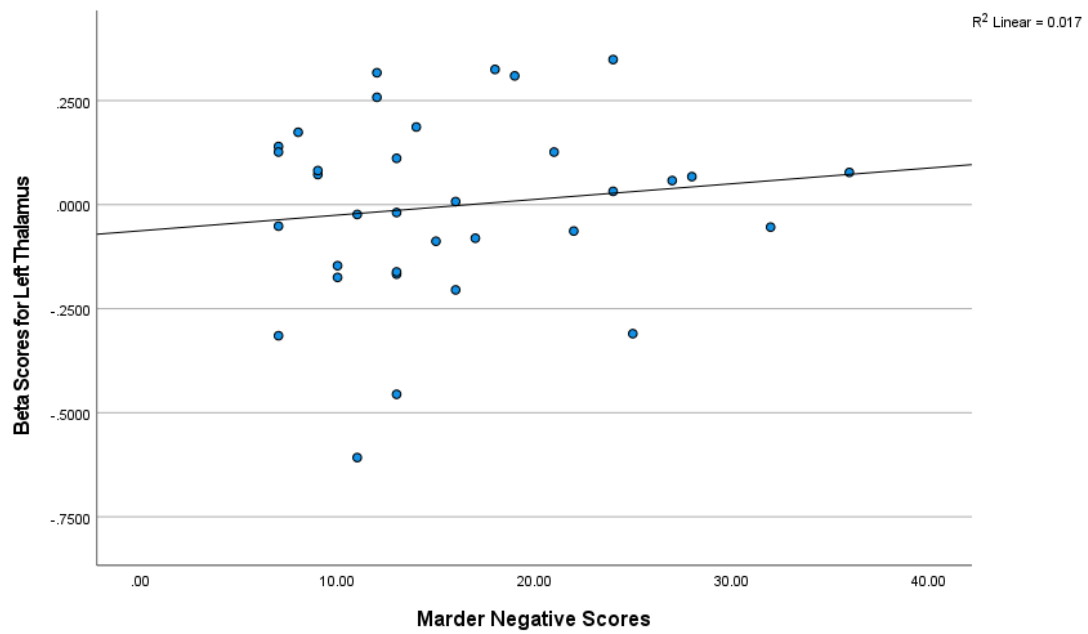
Figure 31: Correlations with Marder Negative Symptoms and Beta Values from Left Thalamus
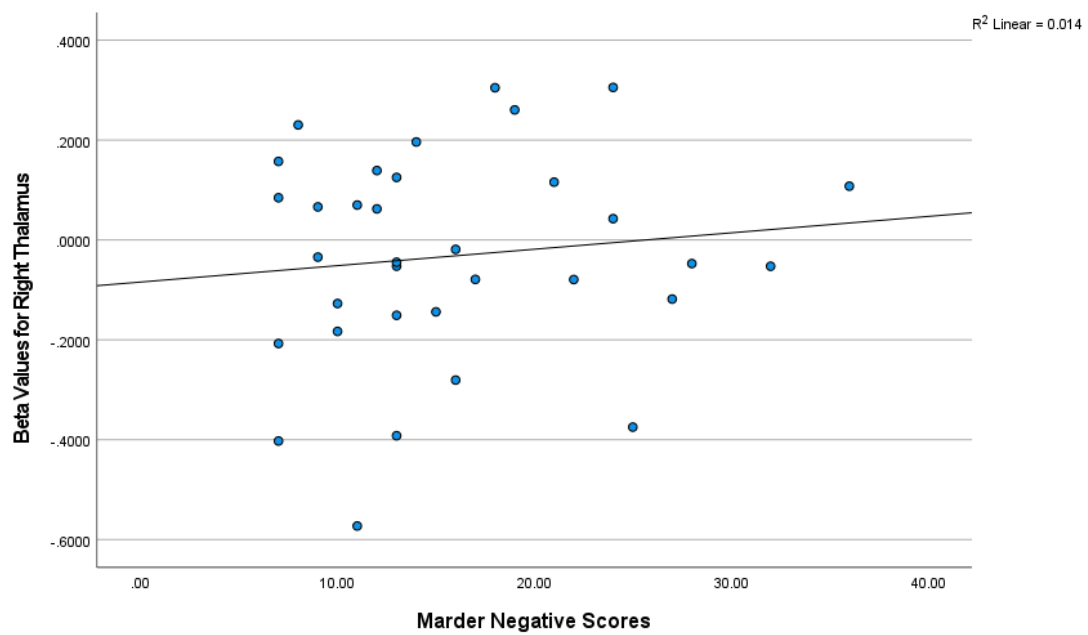
Figure 32: Correlations with Marder Negative Symptoms and Beta Values from Right Thalamus
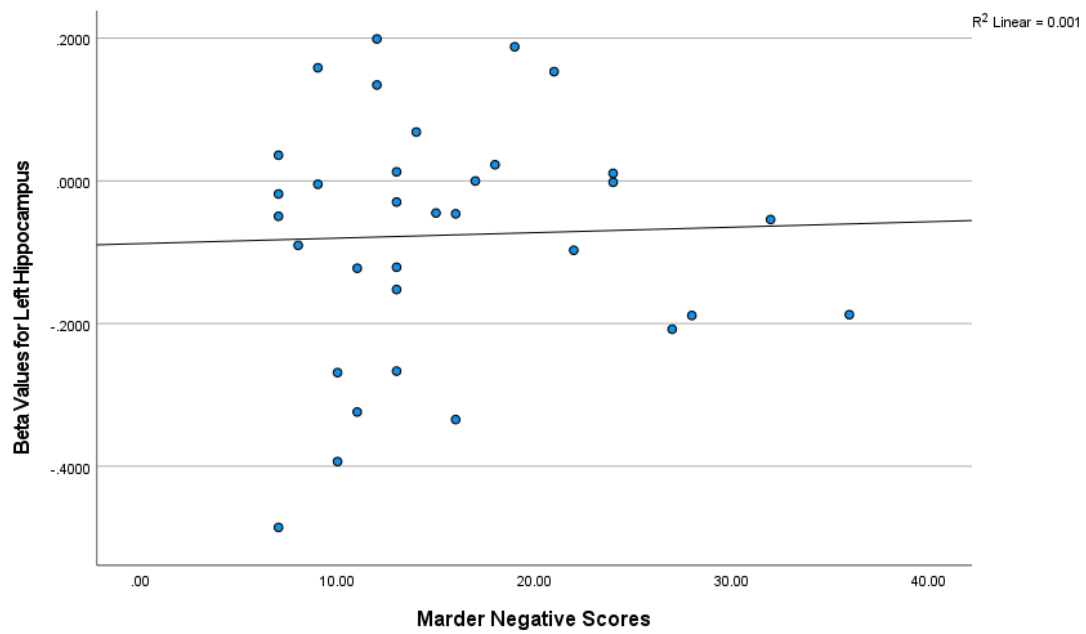
Figure 33: Correlations with Marder Negative Symptoms and Beta Values from Left Hippocampus
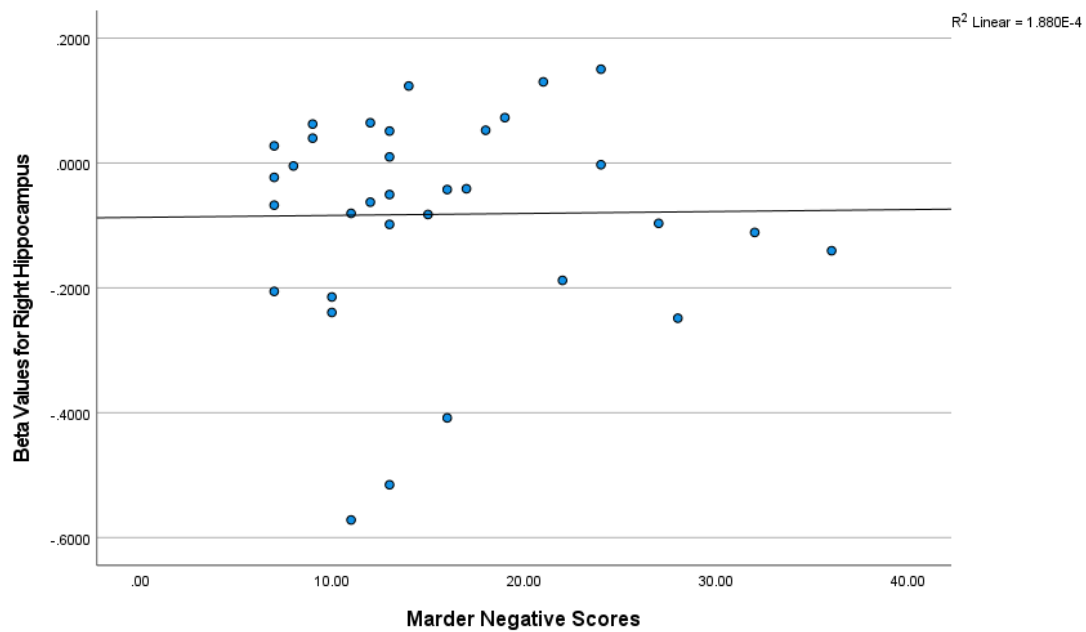
Figure 34: Correlations with Marder Negative Symptoms and Beta Values from Right Hippocampus

2.5. Discussion

First episode psychosis patients showed reduced activation in the thalamus and hippocampus compared to healthy controls during working memory load, which survived the Bonferroni correction. There was no significant group difference in activation in the DLPFC. There were no significant correlations between negative symptom scores and BOLD response during the N-Back task in DLPFC, hippocampus or thalamus. When comparing patients with healthy controls with an exploratory post-hoc group-level analysis using the 2-Back>1-Back contrast, including age, gender, and 2-back performance as regressor of no interest, there was less activation in the hippocampus and thalamus ROIs compared to when no nuisance covariates were included. Although they survived familywise error correction ($p_{FWE}<0.05$), they did not reach the more conservative threshold based on the Bonferroni correction for the number of independent ROIs used (i.e., $p_{FWE}<0.0167$) as they had done in the original analysis.

My findings of reduced activation in the hippocampus and thalamus are in accordance with previous findings in first-episode patients [457, 476] and [294, 296] respectively, and extend them to show this effect in drug free first episode patients for the first time in the hippocampus. My findings contradict the thalamic hyperactivity seen in a previous study of first episode patients compared to healthy controls [437]. However, in this prior study all nine patients [437] were medicated (as they were in a number of other studies [457]) which may affect fMRI responses and signal [272, 273] (but see experimental chapter 4 which disputes this). There has also been one report of hyperfunction in the DLPFC during working memory [461], but, as these patients were medicated, this could reflect a treatment effect. Although the results from Schneider et al (2007) [457] demonstrated hypoactivation in the thalamus and hippocampus, they used (0-back versus baseline) contrasts that it could be argued did not tap working memory but rather attention and basic motor responses.

The failure to detect significant group differences in activation of the dorsolateral prefrontal cortex in first episode psychosis patients is in agreement with one other study [457], but is in disagreement with four previous reports of hypofunction [294, 296, 479, 481]. The reason for the discrepancy with my findings could be that none of these latter four studies controlled for performance on the task, so the results might be due to participants' not performing the task correctly rather than activation differences during working memory performance. Thus, my results extend previous findings by showing that the neural response in the DLPFC is not altered during working memory performance in unmedicated patients. The mean age of patients in this study (25.15 [4.74] years), is comparable to the studies listed in the PRISMA literature review section, see Table 10. In the current study 82% of the patients were male (24 male patients and 5 female patients), which again is comparable to the studies in the PRISMA literature review (Table 10). The mean duration of untreated illness in the current study was 23.33 [18.35] months. Only three studies in the PRISMA review reported the duration of illness as 2.90 years, 28.2 months, or 283 days (see Table 10).

Table 10: Age, Gender, and Illness Duration of Studies Included in Prima Literature Review (in Thesis Introduction)

Study	Mean Age (years) [S.D]	Gender (m/f)	% Male	Illness Duration
Nejad et al. (2011) [474]	26.18 [5.02]	18/5	78%	Not reported
Mendrek et al (2004) [294]	30.00 [8.00]	6/2	75%	Not reported. *
Meisenzahl et al. (2006) [296]	33.50 [9.39]	11/1	91%	Not reported. 29.08 years (age of onset)
Schneider et al. (2007) [457]	31.00 [9.9]	26/22	54%	Not reported
Guerrero-Pedraza et al. (2012) [476]	25.93 [5.82]	21/9	70%	No longer than 18 months
Smieskova et al (2012) [479]	28.57 [7.2]	16/5	76%	2.90 [2.84] years since presentation
Vogel et al. (2016) [461]	28.40 [7.3]	22/0	100%	28.2[38.8] months
Crossley et al. (2009) [481]	Not reported	Not reported	n/a	Not reported
Nielsen et al. (2017) [482]	23.71 [6.89]	10/7	59%	283.0 [145.5] days (course of disease)

* Participants were recruited within one week of being admitted to psychiatric ward

2.5.1.1. Strengths and Limitations:

Strengths of my study include that it was restricted to unmedicated patients, so it is not confounded by current antipsychotic treatment, and performance was considered in the analysis. Even though performance was modelled in the fMRI data analysis, the reader will note that despite only including participants with more than fifty percent correct responses, there were behavioural differences between the groups on the 2-back task. My exploratory findings thus indicate that age, gender and 2-back may contribute to my findings of group differences in activation. Further work is needed to test this, by controlling for age, gender, and performance more stringently in future studies.

Due to the block design of this study, I was not able to investigate different working memory components and sub-processes, although patients with psychosis show different deficits during encoding and retrieval of information [186, 497, 517]. Although the sample is not affected by medication, there is still an issue regarding secondary negative symptoms. It is very difficult to differentiate primary from secondary negative symptoms, especially in a first episode cohort. This may influence the results, as apathy and social isolation caused by anxiety, delusions or avolition a common neurobiological cause. Although it was not possible to differentiate this in the present data.

It is important to recognise the possibility of a type II error explaining the lack of a relationship between neural response and negative symptom ratings. I used the PANSS to assess negative symptoms, in common with previous studies [295, 474]. However it should be recognised that this may not be as sensitive for negative symptoms as scales such as a Clinical Assessment Interview for Negative Symptoms (CAINS) or Brief Negative Symptom Scale (BNSS) [518]. It is also possible a relationship would be detected in patients with more marked negative symptom

severity ratings. Nevertheless, my patient sample showed moderate negative symptoms, like

other comparable first episode studies [295, 461], indicating it is likely to be representative of

patients in general. Moreover, my study was powered to detect a moderate or greater

correlation (r>0.5), and smaller relationships are unlikely to be clinically significant. It is also

important to recognise that the first episode of psychosis groups include patients with affective

psychoses as well as schizophrenia. Thus, my findings are not specific to schizophrenia and

further follow-up is required to determine the final diagnoses in the sample.

Head motion disrupts the MR signal and reduces the quality of the scan [519]. Scripts were used

to calculate frame-wise displacement to identify any participants where head movement was

greater than my threshold of 0.5mm. I removed participants' scans from further analysis if there

were more than 5 additional movement columns/spikes over the duration of the task. Despite

this, participants still made more small movements during the course of a scan. The comparison

of head movement between people with first episode psychosis and healthy controls revealed a

significant difference between the groups. This is a limitation of my data. One way to reduce

motion effects in future studies would be to prospectively coregister all the images in the fMRI

time-series, modifying the scanner to track shifts in the position of the brain as they happen.

There are several methods that have been developed to do this, from the use of external markers

placed on the volunteer's head [520, 521], to methods that calculate rigid-body transformations

of the EPI image, comparable to algorithms used in retrospective motion correction [522], to

techniques that measure differences in k-space [523]. These techniques may reduce the need to

correct for motion retrospectively by making sure that the time-series of EPI images is

coregistered at the time of acquisition. This would thereby minimize signal distortions and

changes in signal-to-noise ratio (SNR) due to motion to reduce the potential of group differences

in motion artifact causing differences in signal strength between the groups.

2.5.1.2.   Implications for understanding the neurobiology of psychotic disorders:

Marked working memory impairments are seen from onset of psychotic disorders and show limited response to current treatments [524]. My findings indicate that patients with psychosis show blunted neural responses in the hippocampus and thalamus in response to increasing working memory load, and this adds to evidence of functional and structural changes in these regions in psychosis [525-528] [382, 529]. This identifies hippocampal and thalamic function as potential targets for the development of treatments to improve working memory performance in psychosis. Further work is required to determine the nature of neurobiological alterations that could be targeted, although other work indicates that glutamatergic and GABAergic alterations could be involved [525, 527, 530].

My findings also indicate that the neurobiology underlying working memory impairments in psychosis is not linked to negative symptoms, arguing against a common pathoetiology. Hypoactivation might reflect less effective recruitment of neurons during the working memory task in patients compared to healthy controls. In the oddball paradigm in patients with schizophrenia, this was postulated to result from interrupted processing of and response to task-relevant stimuli because of the inability to neglect task-irrelevant inputs [531]. It may be that a similar mechanism is at play during the N-Back task in schizophrenia.

2.5.2.   Conclusion

My findings provide evidence that working memory load-related changes in hippocampal and thalamic activation is diminished in first episode psychosis patients relative to controls. This builds on the evidence that these regions are involved in the pathophysiology of psychosis and specifically suggestions that alterations in these regions are involved in working memory impairment.

Table 11: CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	N/A
	1-Back	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	102
Introduction			
Background and	2a	Scientific background and explanation of rationale	103
objectives	2-Back	Specific objectives or hypotheses	103

Methods

Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	105
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	N/A
Participants	4a	Eligibility criteria for participants	105
	4b	Settings and locations where the data were collected	106
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	106
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	107
	6b	Any changes to trial outcomes after the trial commenced, with reasons	N/A
Sample size	7a	How sample size was determined	N/A
	7b	When applicable, explanation of any interim analyses and stopping guidelines	N/A
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	N/A

	8b	Type of randomisation; details of any restriction (such as blocking and block size)	N/A
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	N/A N/A
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	N/A N/A
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	N/A N/A
	11-Back	If relevant, description of the similarity of interventions	N/A
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	114
	12-Back	Methods for additional analyses, such as subgroup analyses and adjusted analyses	114

Results

Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	105
	13b	For each group, losses, and exclusions after randomisation, together with reasons	105
Recruitment	14a	Dates defining the periods of recruitment and follow-up	N/A
	14b	Why the trial ended or was stopped	N/A
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	115
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	115
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	119
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	N/A
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	135
Harms	19	All-important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	N/A

Discussion

Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	143
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	145
Interpretation	22	Interpretation consistent with results, balancing benefits, and harms, and considering other relevant evidence	145

Other information

Registration	23	Registration number and name of trial registry	N/A
Protocol	24	Where the full trial protocol can be accessed, if available	N/A
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	N/A

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items.

If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological

treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up-to-date references relevant to this checklist,

see www.consort-statement.org.

3. Cortical brain function and its relationship with striatal dopamine function in psychosis: a multi-modal MR and PET imaging study

3.1. Abstract

Background: People with schizophrenia show impairments in working memory performance and altered brain activation in a network including the dorsolateral prefrontal cortex (DLPFC), hippocampus and thalamus during working memory tasks, and striatal dopamine dysfunction. However, it is unknown if these alterations are related at the onset of the disorder.

Methods: Twenty-four first episode patients and 18 healthy-matched control participants underwent functional magnetic resonance imaging to index the blood oxygen level dependent (BOLD) response whilst performing a working memory task and also received [^{18}F]DOPA PET imaging to index striatal dopamine synthesis capacity (Ki^{cer}).

Results: Patients showed lower activation in the hippocampus (p = 0.034) and thalamus (p = 0.030) relative to controls. There were no significant differences between the groups in the whole brain analysis. There were no significant differences in dopamine synthesis capacity between the groups. Finally, there were no significant correlations between BOLD response to the N-Back working memory task and Ki^{cer} in either group or between the groups.

Conclusion: Patients show lower BOLD responses in hippocampus and thalamus relative to healthy controls, indicating that altered function in these regions contributes to working memory impairments, but this is not related to striatal dopamine synthesis capacity.

3.2. Introduction

It is not known if the pathophysiology underlying psychosis and cognitive impairments in schizophrenia and associated conditions are related (Howes and Murray 2014). Working memory (WM), the capacity to retain information in mind for short periods of time, is a key cognitive function required for many day-to-day tasks and is commonly impaired in psychotic disorders [400, 443, 444], including from illness onset [445-447] (see 1.6.3.4 . Studies of working memory show it engages a network of brain areas including the dorsolateral prefrontal cortex (DLPFC), hippocampus and thalamus [318, 347, 350, 351, 407, 458]. In general, the findings of brain responses during working memory tasks in first episode patients show hypofunction in the DLPFC, thalamus and hippocampus [294, 413, 461, 496-499], although hyperfunction[481] and no group differences have also been reported [457, 476] (see 1.6.3.4 PRISMA Checklist). These inconsistencies may be due to methodological differences studies, in particular, whether the attentional control or lower working memory load condition is used for the contrast [294, 296, 457, 461, 476, 479, 481, 482, 501], and/or the inclusion of patients taking antipsychotics, which may affect functional MRI signal [277, 280, 500] (but see experimental chapter 4 which disputes this).

Positron emission tomography (PET) studies, using [18F]DOPA and [11C]L-DOPA ligands, have investigated the nature of dopamine dysfunction in schizophrenia. The majority of these studies show an elevation of striatal dopamine synthesis capacity in people at ultra-high risk (UHR) of developing psychosis [136-142], first episode patients [145-148] and chronic patients [149, 150] compared to healthy matched controls. Based on tracing studies, the striatum can be subdivided into sensorimotor, associative, and limbic sub-divisions based on these topographical studies [103, 166, 167]. The associative striatum receives projections from prefrontal cortical regions

[136, 137, 169-171]. PFC dysfunction might lead to striatal dopamine dysfunction [172] or might be the consequence of DLPFC abnormalities in schizophrenia [173] (see 1.3.3.1).

In rodent models and in patients with schizophrenia, hippocampal hyperfunction is thought to contribute to dopamine hyperfunction [83], leading to cognitive impairment. The hippocampus sends projections to ventral (limbic) striatum subdivision, which activates midbrain dopamine neurons which then project back to the associative striatum (AST) [532]. This might explain why psychosis has been associated with hyperdopaminergia in the AST [144]. Lisman and Grace propose that elevated dopamine in the striatum is driven by medial temporal lobe (MTL) dysfunction [72].

To date, few studies have combined fMRI and PET to look at the relationship with working memory in the DLPFC or MTL and striatal dopamine in patients. In the at-risk population, increased striatal dopamine synthesis capacity was correlated with decreased prefrontal cortex activation [139, 140], and a positive correlation has been reported between BOLD activation in MTL clusters and striatal dopamine synthesis capacity [144]. In chronic patients, higher striatal dopamine uptake was associated with lower PFC activation levels in patients, but not controls during a Wisconsin Card Sorting Task (WCST) [326]. Thus, studies in both at risk and chronic patients indicate a link between striatal dopamine and cortical neural responses during cognitive tasks. However, it is not known if cortical activation is related to striatal dopamine in first episode psychosis patients.

Based on the previous studies, I hypothesised that the treatment-free first episode psychosis patients would have reduced dorsolateral prefrontal cortex, hippocampal and thalamic activity

during the working memory tasks relative to healthy controls, and this would be associated with greater striatal dopamine function.

(Intentional Break)

3.3. Methods

3.3.1. Study design and population sample

Please see p.105 (section 2.3.1) for details.

3.3.1.1. Patient Group (n = 24)

45 patients, who presented to first episode psychosis services, were recruited into the study. 24 patients had PET scans and 45 had MRI scans. Of this number, 24 had both an MRI and PET scan and were included in the study. Please see p.105 (section 2.3.1.1) for further details.

3.3.1.2. Healthy Control Group (n = 18)

33 healthy controls were recruited into the study. 18 had PET scans and 33 had MRI scans. Of this number, 18 had both MRI and PET scans were included in this study. Please see p.106 (section 2.3.1.2) for further details.

3.3.1.3. Exclusion criteria for all participants:

Please see p. 106 (section 2.3.1.3) for details.

3.3.2. Clinical Measures

Consumption of illicit substance, alcohol and tobacco were evaluated using a modified Cannabis Experiences Questionnaire [533]. As in chapter 2, the PANSS, NART and GAF were employed (please see P. 106, section 2.3.2 for further details).

3.3.3. fMRI scanning

3.3.3.1. Image acquisition

Please see p.107 (section 2.3.3.1) for details in chapter 2.

3.3.4. PET Scanning

3.3.4.1. Image Acquisition

All participants were asked not to eat or drink (except water), and refrain from alcohol for 12 hours prior to scanning. Cigarette smokers were not permitted to smoke for four hours preceding the scan [534]. Imaging data from the PET scans were obtained on a Siemens Biograph 6 HiRez PET scanner (Siemens, Erlanger, Germany) in three-dimensional mode. One hour before the scan, participants were given 400 mg entacapone, a peripheral catechol-0-methyltransferase inhibitor, and 150 mg carbidopa, a peripheral aromatic acid decarboxylase inhibitor, to strengthen specific signal detection. These compounds decrease the formation of radiolabelled metabolites that may cross the blood-brain barrier [535, 536]. A 10-minute transmission scan was conducted before the radiotracer injection using approximately 150-MBq of ^{18}F-DOPA was administered by bolus intravenous injection 30 seconds after the start of the PET imaging. Participants were positioned in the scanner with the orbitomeatal line parallel to the transaxial plane of the tomograph. Head position was marked, monitored and movement minimized using a head strap. Radiolabelled L-DOPA is converted to dopamine by DOPA decarboxylase. ^{18}F-DOPA PET measures the rate constant for ^{18}F-DOPA uptake, transport to synaptic vesicles and conversion into ^{18}F-dopamine in dopamine neurons, thereby providing a measure of dopamine synthesis capacity [537, 538]. PET data were acquired in 32 frames of increasing duration over the 95 min scan (frame intervals: 8 × 15 s, 3 × 60 s, 5 × 120 s, 16 × 300 s).

3.3.5. Statistical analysis

The effect of group on demographics, clinical measures, Ki^{cer} values and BOLD signal (in SPM) were tested using independent t-tests or analysis of variance for parametric variables. The Mann-Whitney U tests were used for non-parametric variables after checking for the equality of variance using the Levene test. The Chi-squared test was used for categorical data. To identify outliers in the correlations, boxplots were used. A mixed factorial 3x2 ANOVA was used to analyse the N-Back behavioural scores where the three levels of the N-Back task (2-back, 1-back, and 0-back) were the within-subject factors, and group (patient or healthy control) was the between-subjects factor.

3.3.6. fMRI image analysis

To test the hypothesis that there were between-group differences in activation, I performed a second-level analysis comparing activation during N-Back, for each of the contrasts between the 2 groups, using independent-samples t-tests. For details of pre-processing and first level pre-processing, please see p.108, section 2.3.3.2.

In the second level analysis, a 2x2 mixed-design ANOVA was used to test for a load-by-group interaction, with 1-Back>0-Back and 2-Back>0-Back as the levels. The N-Back behavioural scores were used as covariates to address the issue of performance differences between the groups. I employed a region-of-interest analysis approach. A priori ROI masks were generated for the hippocampus and thalamus bilaterally using the AAL Atlas in WFU Pick-Atlas *[514]*. The DLPFC ROI was also created in WFU Pick-Atlas *[514]* using TD Brodmann Areas + (comprised of BA9 and BA46 [318, 515, 516]). In all cases, unless otherwise stated, a result was deemed significant if it survived family-wise error (FWE) correction based on cluster extent (FWE<0.05) within the whole brain or

peak (s.v.c FWE<0.05) predefined small volumes (i.e., ROI masks) using the SPM default uncorrected height threshold of p<0.001.

3.3.7. PET Image Analysis

Head movement in the scanner was adjusted for using non-attenuation-corrected dynamic images denoized by using a level 2, order 64 Battle-Lemarie wavelet filter. Nonattenuation-corrected images used the realignment algorithm [539]. Frames were realigned to a single 'reference' frame, which was acquired 20 minutes post-injection, using a mutual information algorithm [540]. The transformation parameters were applied to the corresponding attenuated-corrected dynamic images. The realigned frames were then summated, creating a movement-corrected dynamic image, which was used in the analysis.

Realigned frames were then summated to create an individual motion-corrected reference map for the brain tissue segmentation. SPM8 (http://www.fil.ion.ucl.ac.uk/spm) was used to normalize a tracer-specific ([^{18}F]-DOPA) template [136, 541] together with the HamNet probabilistic brain atlas [542] both in the same space to each individual PET summation image. The brain atlas was used to identify the whole striatum, functional striatal subdivisions and the cerebellar region used as reference for tissue quantification [542]. The HamNet brain atlas was used to identify the whole striatum and the cerebellar region used as the reference for tissue quantification [542]. The striatal influx constant (K_i^{cer} [1/min])[58] written as K_i in some previous publications [136]) was calculated compared with uptake in the reference region using a graphical approach adapted for a reference tissue input function [541]. A previous test/re-test study has shown this approach has good reliability [541]. The cerebellar reference region [538] was defined using a probabilistic atlas [542], and regions of interest (ROI) in the whole striatum and its

functional sub-divisions were delineated to create an ROI map as previously described [541].

Further details of the image analysis approach are given in Bloomfield et al [534]. Dopamine

synthesis capacity (ki^{cer}) was calculated using the Patlak-Gjedde graphical approach adapted for a

reference tissue input function[543] . This method has been shown to have good reliability for

measuring Ki^{cer} (intra-class correlation coefficients >0.85 for striatal ROIs) [541]. The cerebellar

region was used as a reference region as it is has been revealed to have no or little specific

[^{18}F]fluorodopa uptake [544] and has negligible dopamine projections [545, 546]. It therefore

provides an index of non-specific uptake of the tracer, which is then used to calculate the uptake

and conversion of F-DOPA to F-Dopamine in the striatum. SPM was used for co-registration of the

images.

3.3.8.   Integration of fMRI and PET data

Two-tailed Pearson's correlations were conducted between hippocampus and thalamus during the

working memory task and striatal Ki^{cer} values from the PET analysis. β values were extracted using

MarsBar 0.44 SPM toolbox (http://marsbar.sourceforge.net/) and were plotted against Ki^{cer} values

for the whole, left and right associative striatum. Fisher's r-to-z transformations (two-tailed) were

used to investigate the difference in correlation r-values between patients and controls using the

following equation:

$$Z\ difference = \frac{Zr_1 - Zr_2}{\sqrt{\frac{1}{N_1-3}+\frac{1}{N_2+3}}}$$

3.4. Results
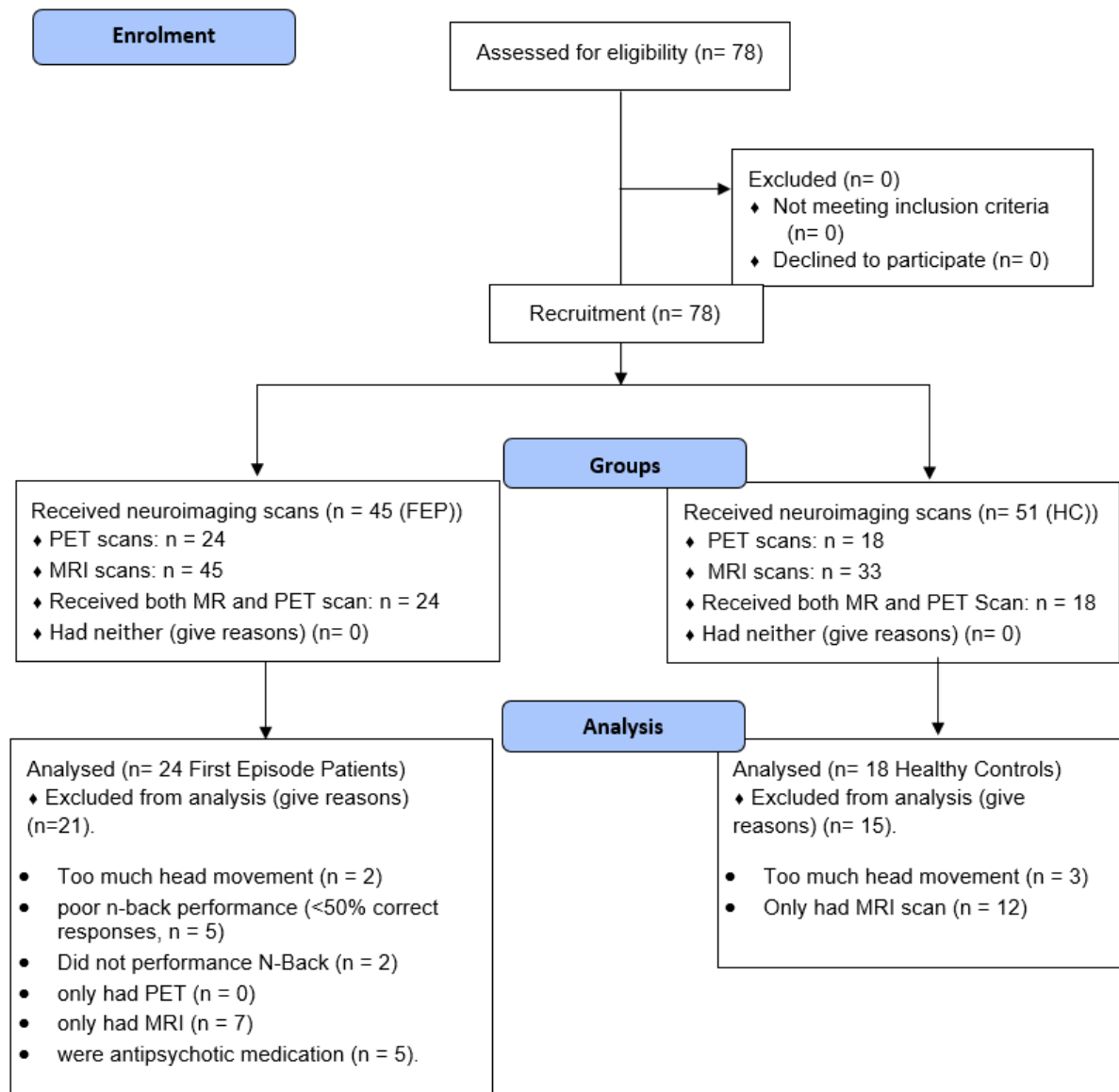
Figure 35: CONSORT 2010 Flow Diagram

Clinical and demographic characteristics of the sample can be found in Table 12. There were no significant differences between the groups in terms of age, handedness, or ethnicity. The two groups differed significantly with regards to gender (p = 0.047). Patients had a mean duration of untreated illness of 25.06 [19.00] months. 10 patients were treatment-naïve and 14 were treatment-free. 13 patients had a diagnosis of schizophrenia, 9 were diagnosed with affective bipolar disease and 2 with psychotic depression. The total mean PANSS score was 69.81 [22.29]. See Table 12 for positive, negative, general, and total PANSS scores for patients. Patients had a median number of 7 days between PET and MRI scans, whereas healthy controls had a median of 58 days between scans.

There was a significant main effect of load on N-Back performance ($F_{(2,80)}$ = 7.199, p = 0.001) whereby the N-Back scores significantly differed between each level regardless of group. There was a significant main effect of group (see Table 13, $F_{(1,40)}$ = 6.659, p = 0.014)) on behavioural scores on the N-Back task. These refer to the number of correct responses participants obtained out of 12 for each level of the N-Back task. Bonferroni corrected post-hoc tests showed that the scores of 2-back, 1-back and 0-back did not differ significantly (p = 0.021, p = 0.107 and 0.420 respectively - see Table 13 and Figure 36). There was a no significant N-Back x group interaction, $F_{(2,80)}$ = 2.510, p = 0.088. There was no significant difference in injected activity (p = 0.069), nor dopamine synthesis capacity between groups in the associative striatum (p = 0.962) or limbic striatum (p = 0.808).

Table 12: Clinical and demographics variables

Variable	Healthy controls (n=18)	Patients (n=24)	Statistics
Sex (female: male)	7:11	3:21	χ2 (1) = 3.948, p = 0.047
Age [SD] years	23.6 [3.74]	25.2 [4.32]	t(40) = 1.165, p = 0.251
Ethnicity			χ2 (4) = 8.099, p = 0.088
White British/Other	13	10	
Black British/Other	1	9	
Asian British/Other	2	2	
Other	1	3	
Handedness (% R)	66.7%	75%	χ2 (3) = 2.256, p = 0.521
Injected Activity (mBq)	152.44 [12.78]	145.18 [12.17]	t(40) = 1.186; p = 0.069
Associative Striatum	0.013 [0.001]	0.013 [0.001]	t(40) = 0.048, p = 0.962
Limbic Striatum	0.013 [0.001]	0.013 [0.001]	t(40) = 0.244, p = 0.808
Days between scans	median = 58.5	median = 7	n/a
PANSS Positive	n/a	19.06 [6.75]	n/a
PANSS Negative	n/a	15.63 [5.83]	n/a
PANSS General	n/a	35.13 [11.90]	n/a
PANSS Total	n/a	69.81 [22.29]	n/a

DUI = Duration of Untreated Illness; TN = Treatment Naïve; TF = Treatment-Free.
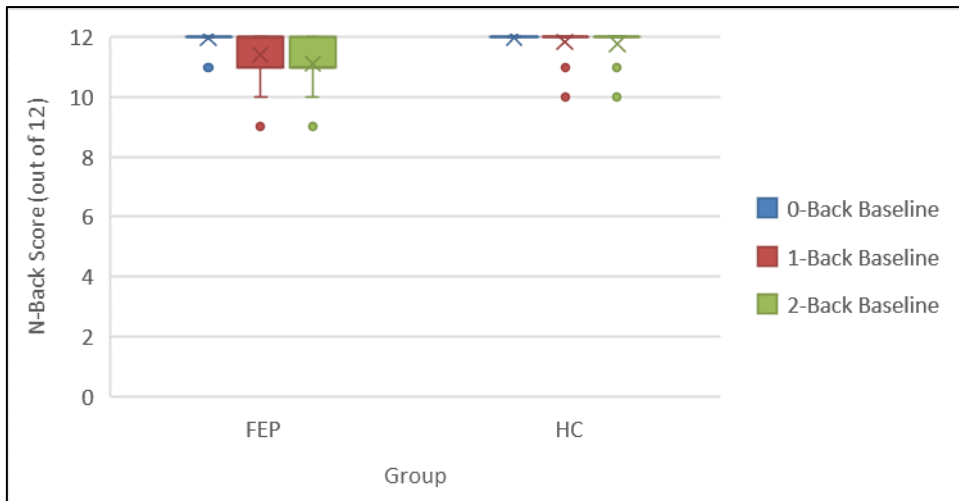
Figure 36: Boxplots of the Mean N-Back Behavioural Scores for Patients with First Episode Psychosis (FEP) and Healthy Controls (HC)

Table 13: Mean [standard deviation] N-Back Behavioural Scores

N-Back	Healthy Controls	First Episode Patients	Bonferroni Corrected t-tests (p<0.0167)
0-Back	12.00 [0.00]	11.96 [0.20]	t(43) = 0.813, p = 0.420
1-Back	11.83 [0.52]	11.46 [0.93]	t(43) = 1.648, p = 0.107
2-Back	11.78 [0.55]	11.08 [1.14]	t(43) = 2.402, p = 0.021

3.4.1. fMRI Results

3.4.1.1. Formal Test of Head Movement

The results show that there was a significant effect of head movement between the groups (t(36) = 2.201, p = 0.034 (see Figure 37)).
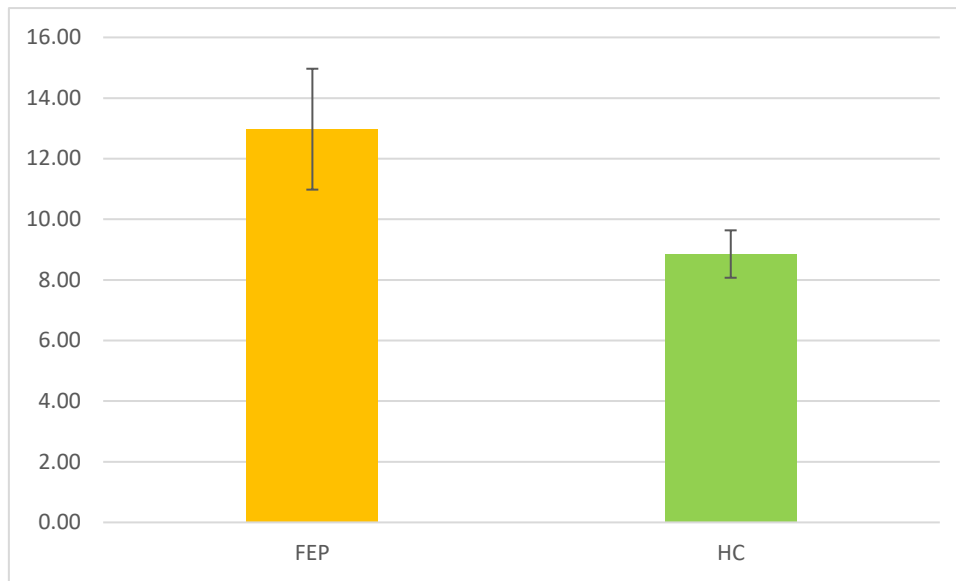
Figure 37: Mean Head Movement for Patients and Healthy Controls (in millimetres)

3.4.1.2.   Main Effect of Task

In healthy control participants, during a higher working memory load compared to a lower working memory load (1-Back<2-Back) there was activation in the precuneus, precentral gyrus, inferior and superior frontal gyri, and superior parietal lobules (see Table 14 and Figure 38). In patients, during a higher working memory load compared to a lower working memory load (1-Back<2-Back) there was activation in the superior frontal gyrus, precuneus, superior parietal lobule and insula (see Table 15 and Figure 39).

Table 14: Foci of Brain Activation during the 2-Back>1-Back N-Back Task in Healthy Controls

Region	Left or Right	MNI Coordinates			No. of Voxels	z-scores	Cluster Level (FWE correction)
		x	y	z			
Superior Frontal Gyrus	Right	26	-2	54	14204	5.78	0.000
Precuneus	Right	16	-64	46	4383	5.36	0.000
Superior Parietal Lobule	Left	-20	-64	54	3581	5.28	0.000
Insula	Left	-30	18	6	478	4.09	0.025
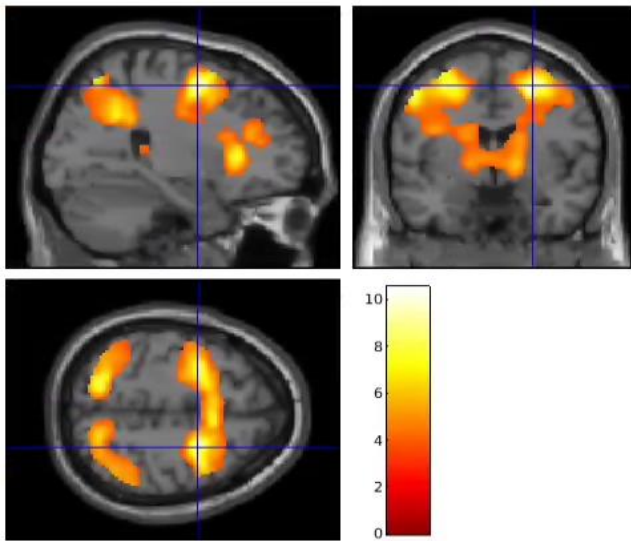
Figure 38: This shows significant foci of brain activation during the 2-Back>1-Back contrast in healthy controls (cluster level pFWE corrected<0.05), whole brain analysis. The peak is shown by the cross hair. The colour bar indicates the t value.

Table 15: Foci of Brain Activation during the 2-Back>1-Back N-Back Task in patients

Region	Left or Right	MNI Coordinates			No. of Voxels	z-scores	Cluster Level (FWE correction)
		x	y	z			
Middle Frontal Gyrus	Right	30	0	54	720	5.78	0.002
Supramarginal Gyrus	Right	44	-38	42	4134	5.57	0.000
Middle Frontal Gyrus	Left	-30	28	32	1676	5.28	0.000
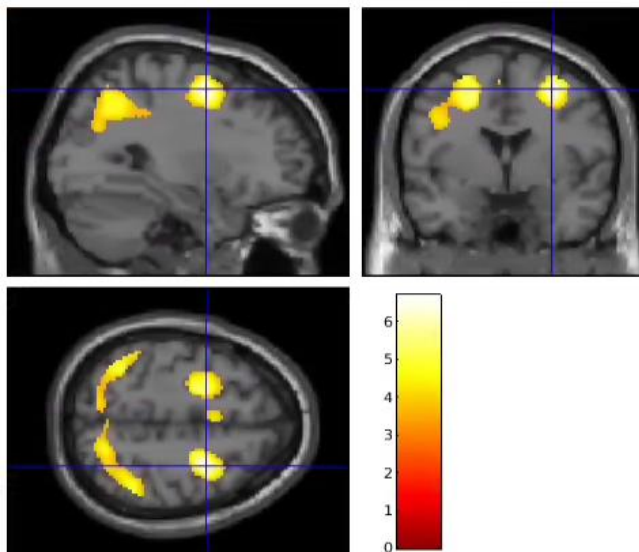
Figure 39: This shows significant foci of brain activation during the 2-Back>1-Back contrast in patients (cluster level pFWE corrected<0.05), whole brain analysis. The peak is shown by the cross hair. The colour bar indicates the t value.

In the whole brain analysis in healthy control volunteers, during a higher working memory load compared to the control, motor task (2-Back>0-back contrast) there was activation in the superior

frontal gyrus, middle frontal gyrus and superior parietal lobule (see Table 16, and Figure 40). In

patients during this contrast, there was activation in the inferior parietal lobule, middle frontal

gyrus and inferior temporal gyrus (see Table 17 and Figure 41).

Table 16: Foci of Brain Activation during the 2-Back>0-Back N-Back Task in Healthy Controls

Region	Left or Right	MNI Coordinates			No. of Voxels	Z- Scores	Cluster Level (FWE correction)
		x	y	z			
Angular gyrus	Right	30	-62	44	3553	5.33	p<0.001
Middle Frontal Gyrus	Right	28	-2	56	5503	5.17	P<0.001
Middle Frontal Gyrus	Right	34	34	26	910	5.14	**0.**004
Superior parietal gyrus	Left	-20	-68	48	2059	4.50	P<0.001
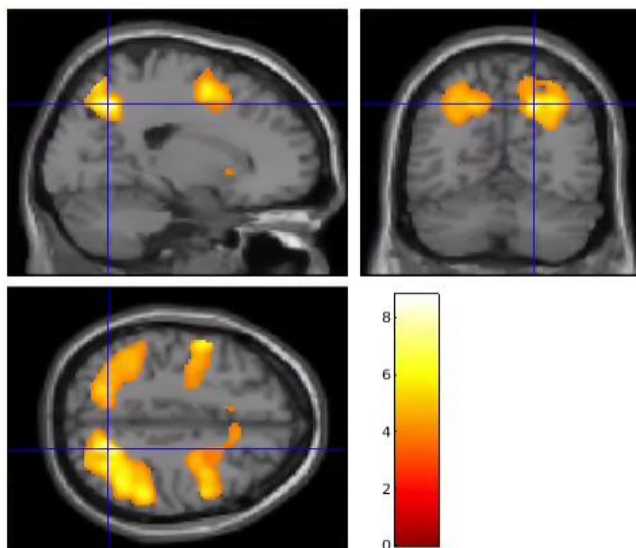
Figure 40: This shows the foci of brain activation during the 2-Back>0-Back contrast in healthy controls (cluster level pFWE corrected<0.05), whole brain analysis. The peak is shown by the cross hair. The colour bar indicates the t value.

Table 17: Foci of Brain Activation during the 2-Back>0-Back N-Back Task in patients

Region	Left or Right	MNI Coordinates			No. of Voxels	Z-Scores	Cluster Level (FWE correction)
		x	y	z			
Inferior Parietal Lobule	Left	-30	-56	40	7157	5.51	p<0.001
Middle Frontal Gyrus	Right	32	0	52	12941	5.46	p<0.001
Inferior Temporal Gyrus	Left	-42	-56	-8	572	4.12	0.007
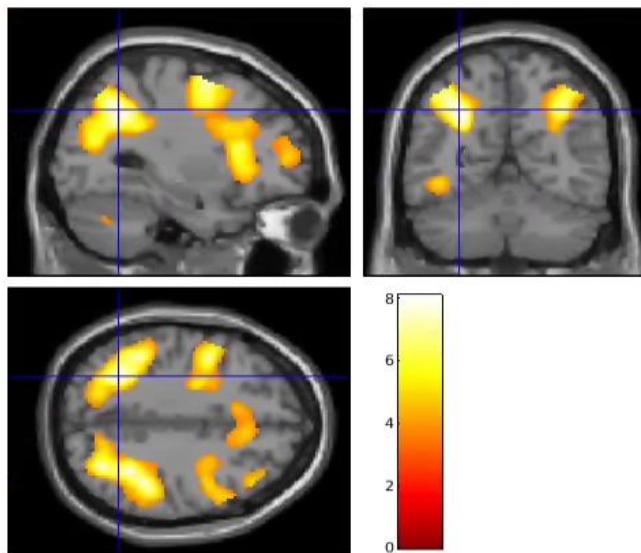
Figure 41: This shows the foci of brain activation during the 2-Back>0-Back contrast in patients (cluster level pFWE corrected<0.05), whole brain analysis. The peak is shown by the cross hair. The colour bar indicates the t value.

3.4.1.3. First Episode Psychosis Patients vs Healthy Controls

In whole-brain analyses (uncorrected height threshold of $p<0.001$) of the two (group) by two (WM load) interaction, there were no suprathreshold clusters for either contrast ((1-Back>0-Back) > (2-Back>0-Back) or (1-Back>0-Back) < (2-Back>0-Back)). The region of interest analysis showed significantly more activation in healthy controls (HC) compared to patients (FEP) when participants performed the 2-back task compared to the 1-back condition in the hippocampus and thalamus (Table 18, Figure 42 and Figure 43). The hippocampus ROI survived the familywise error correction ($pFWE<0.05$) and reached the more conservative threshold based on the Bonferroni correction for the number of independent ROIs used (i.e., $pFWE<0.0167$). There was no significant difference in activation between the groups in the DLPFC. There was no significant activation difference between patients and healthy controls during the whole brain or small volume correction analyses for 2-Back<0-Back contrast.

Table 18: ROI Activation is greater in healthy controls than FEP patients when 2-Back>1-Back conditions were contrasted (height threshold p = 0.001)

ROI	L / R	MNI Coordinates			No. of voxels	z-scores	Peak Level (pFWE-corrected)	FWE error corrected with additional Bonferroni Correction (p<0.0167)
		x	y	z				
Hippocampus	R	22	-34	10	12	3.76	0.014	Significant
Thalamus	R	20	-30	12	16	3.55	0.023	Not Significant
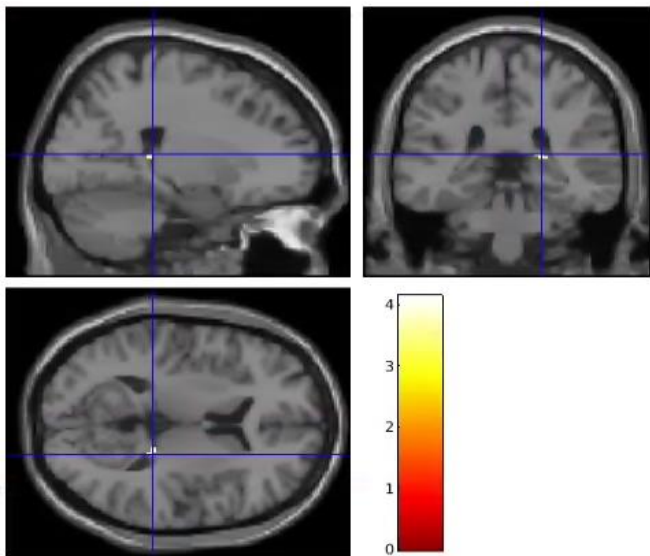
Figure 42: This shows regions of significantly greater activation in healthy control participants relative to patients for 2-back>1-back contrast at baseline in the hippocampus. For hypothesis-led ROI analyses, statistical maps were initially thresholded at an uncorrected height threshold of p<0.001, followed is family-wise error correction via small volume correction (SVC;

pFWE<0.05) using hippocampus ROI. The peak is shown by the crosshair. The colour bar

indicates the t value.
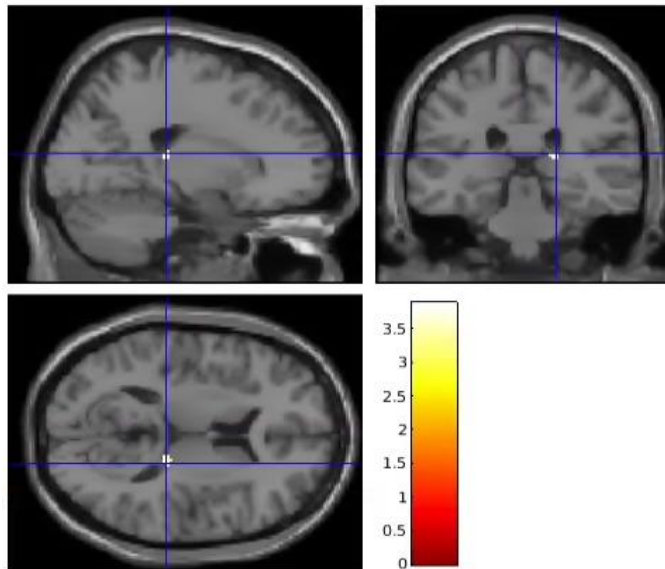
Figure 43: This shows regions of significantly greater activation in healthy control participants relative to patients for 2-back>1-back contrast at baseline in the thalamus. For hypothesis-led ROI analyses, statistical maps were initially thresholded at an uncorrected height threshold of p<0.001, followed is family-wise error correction via small volume correction (SVC; pFWE<0.05) using hippocampus ROI. The peak is shown by the crosshair. The colour bar indicates the t value.
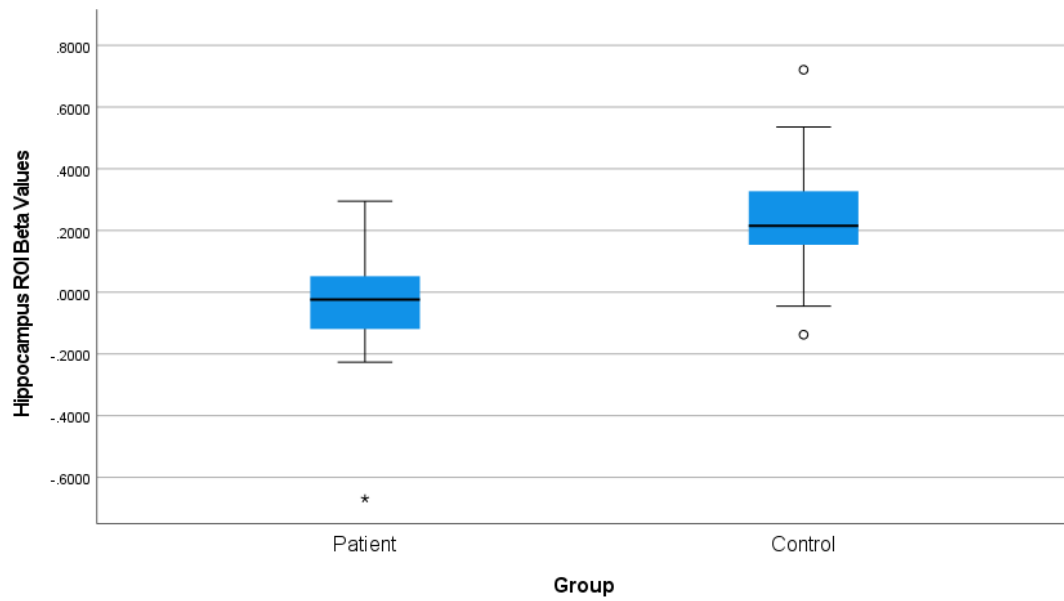
Figure 44: Boxplot of beta value for patients with first episode psychosis and healthy controls, in the hippocampus ROI
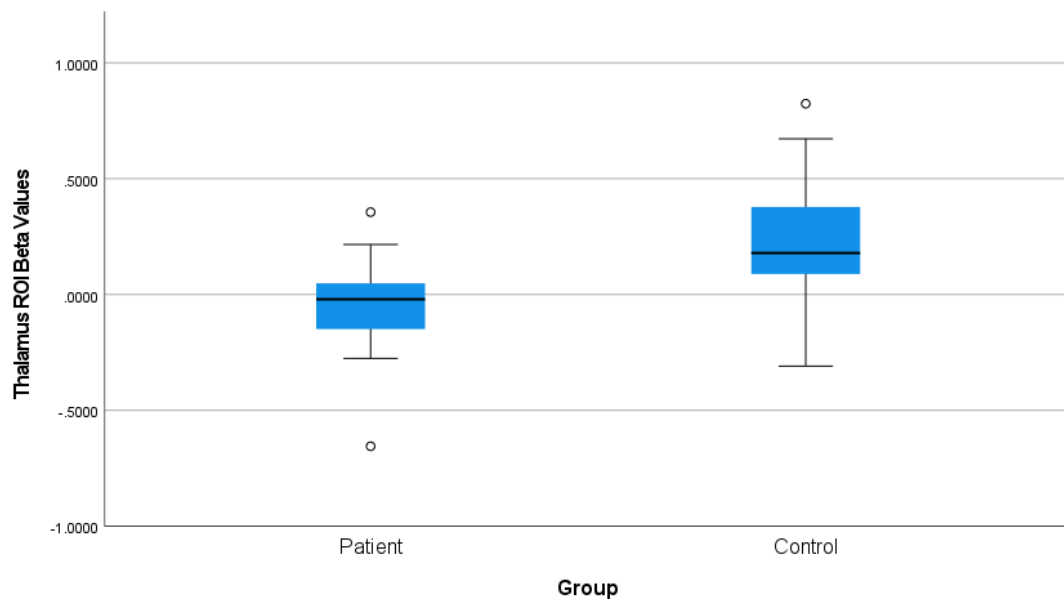
Figure 45: Boxplot of beta value for patients with first episode psychosis and healthy controls, in the thalamus ROI

3.4.1.4. Nuisance Covariates - Age, Gender, and 2-Back Performance

When age, gender and 2-back performance were covaried, in the 2-Back>1-Back contrast there was no suprathreshold clusters in healthy controls compared to patients in the hippocampus, thalamus and DLPFC small volume corrected analysis.

3.4.2. The relationship between dopamine function and BOLD activation (2-Back>1-Back)

For ROIs where there was evidence of significant group differences in activation (i.e., small volume correction), I examined the relationship between mean neural activation in this region and dopamine synthesis capacity (DSC) in the whole associative and striatal regions. There were no significant correlations with DSC and BOLD response in any of the regions of interest in either the patient group or the healthy control group. There were also no statistically different correlations between patients and controls (see Table 19, Figure 46, Figure 47, Figure 48, Figure 49, Figure 50, Figure 52, Figure 51, Figure 52, and Figure 53).

(Intentional break)

Table 19: No significant correlations between dopamine synthesis capacity in the associative striatum and BOLD response in the thalamus and hippocampus (during the 2-Back>1-Back contrast)

	Patients or Controls	Whole Thalamus	Z Difference (Thalamus)	Whole Hippocampus	Z difference (Hippocampus)
Whole Associative Striatum	Patients	r = 0.019, p = 0.930	z = -0.35 p (two-tailed) = 0.726	r = 0.079, p = 0.721	z = -0.07, p (two-tailed) = 0.796
	Controls	r = 0.040, p = 0.880		r = 0.186, p = 0.481	
Whole Limbic Striatum	Patients	r = 0.140, p = 0.513	z = 0.32 p (two-tailed) = 0.749	r = -0.001, p = 0.996	z = -0.35 p (two-tailed) = 0.726
	Controls	r = 0.042 p = 0.868		r = 0.107 p = 0.673	
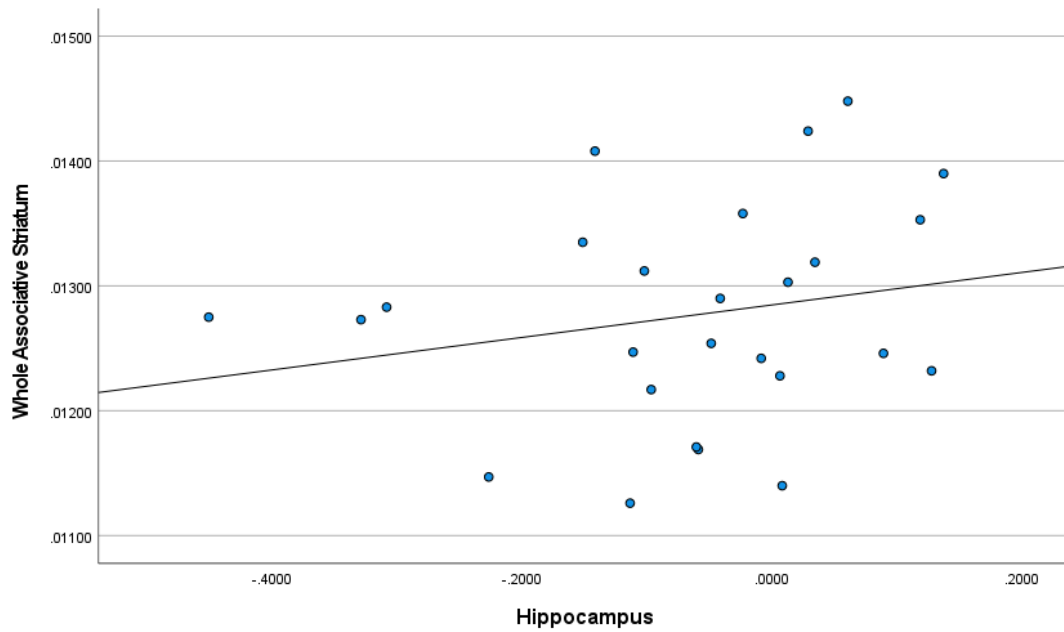
Figure 46: No significant correlations between dopamine synthesis capacity in the whole associative striatum and BOLD response in the hippocampus (during the 2-Back>1-Back contrast) in patients
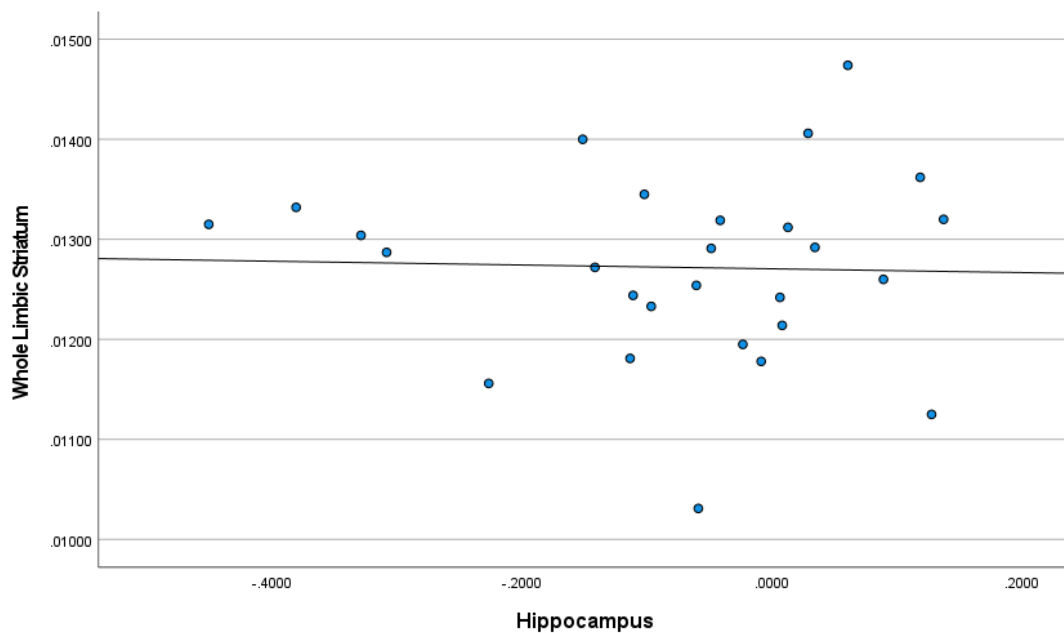
Figure 47: No significant correlations between dopamine synthesis capacity in the whole limbic striatum and BOLD response in the hippocampus (during the 2-Back>1-Back contrast) in patients
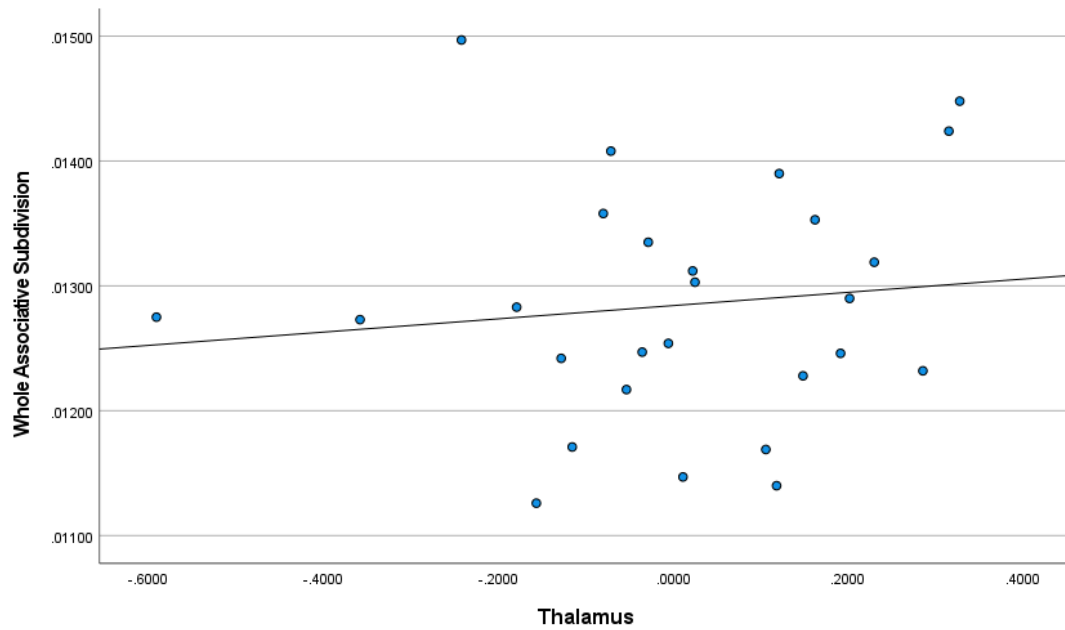
Figure 48: No significant correlations between dopamine synthesis capacity in the whole associative striatum and BOLD response in the thalamus (during the 2-Back>1-Back contrast) in patients
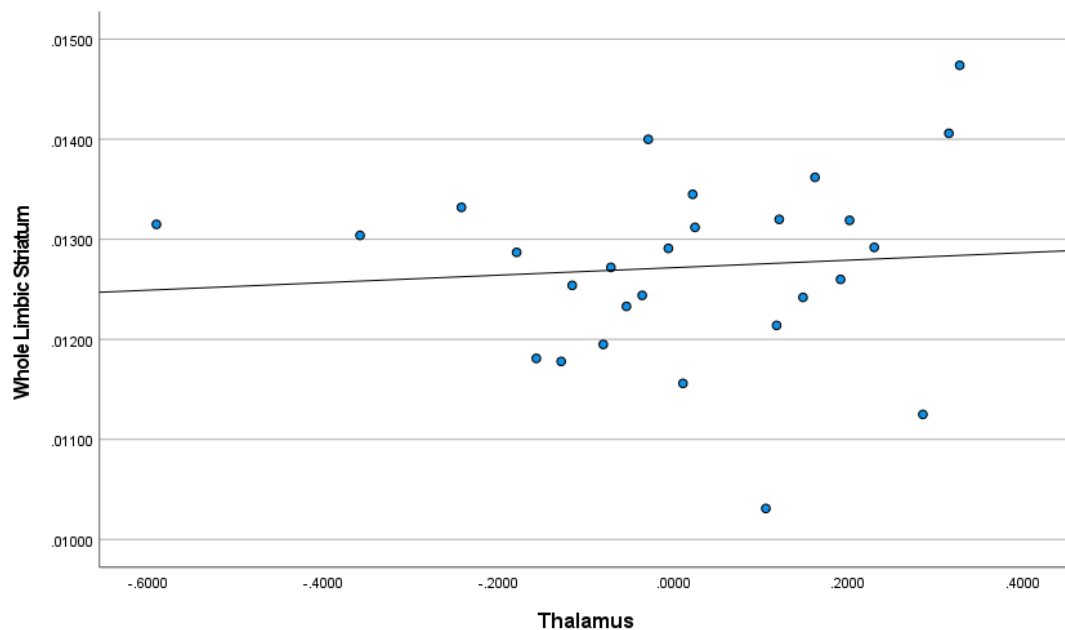
Figure 49: No significant correlations between dopamine synthesis capacity in the whole limbic striatum and BOLD response in the thalamus (during the 2-Back>1-Back contrast) in patients
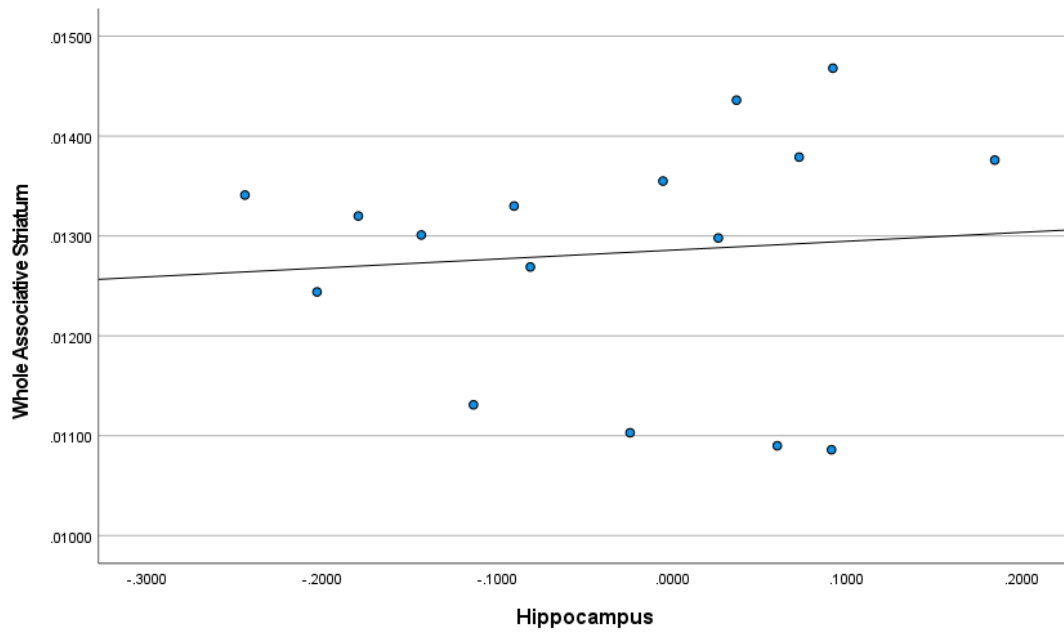
Figure 50: No significant correlations between dopamine synthesis capacity in the whole associative striatum and BOLD response in the hippocampus (during the 2-Back>1-Back contrast) in healthy controls
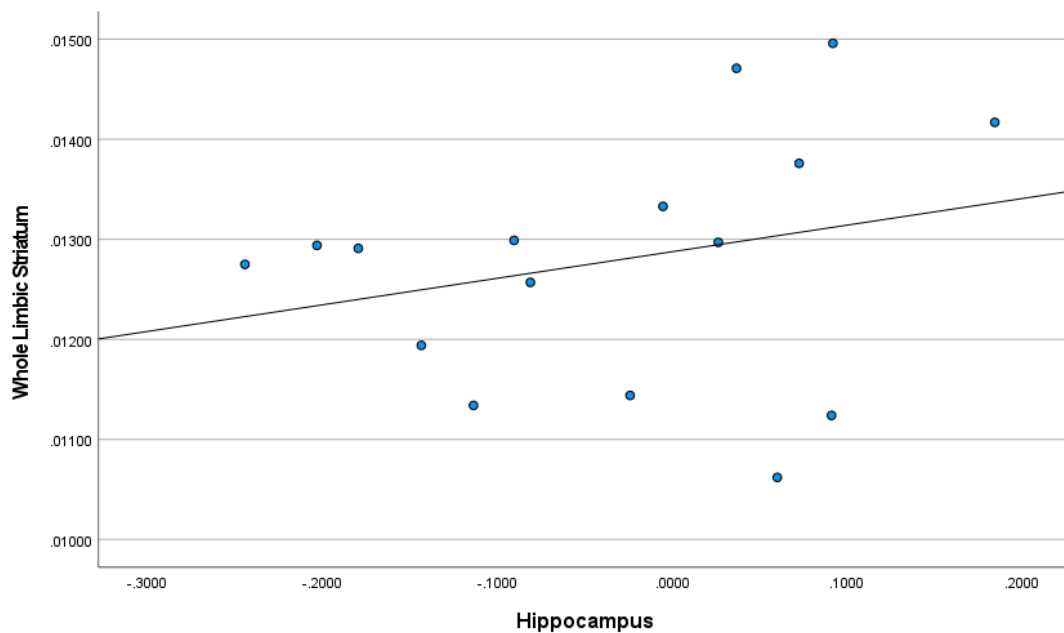
Figure 51: No significant correlations between dopamine synthesis capacity in the whole limbic striatum and BOLD response in the hippocampus (during the 2-Back>1-Back contrast) in healthy controls
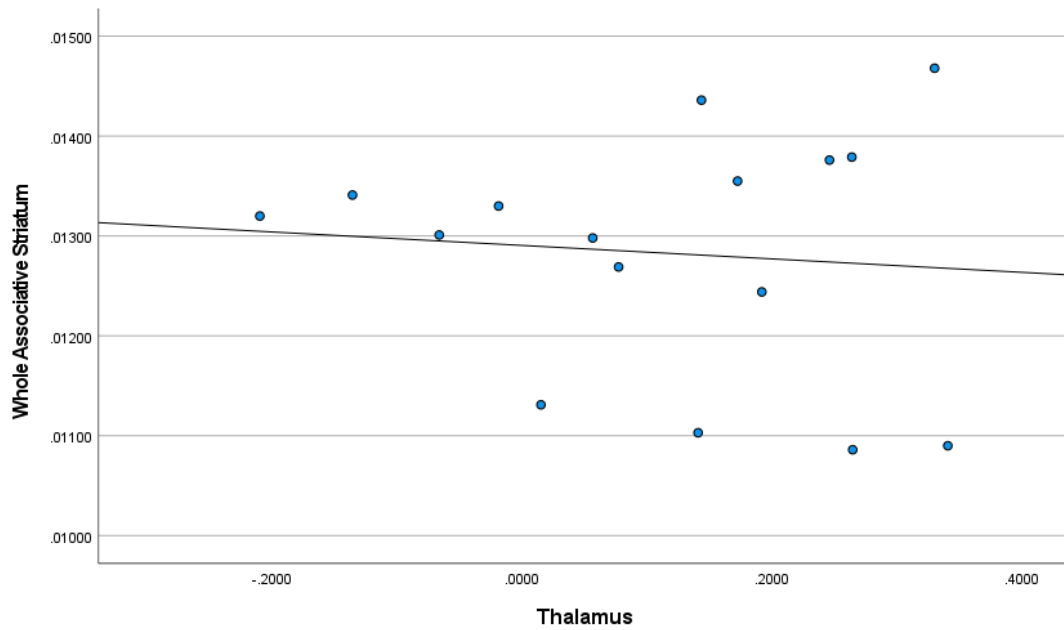
Figure 52: No significant correlations between dopamine synthesis capacity in the whole associative striatum and BOLD response in the thalamus (during the 2-Back>1-Back contrast) in healthy controls
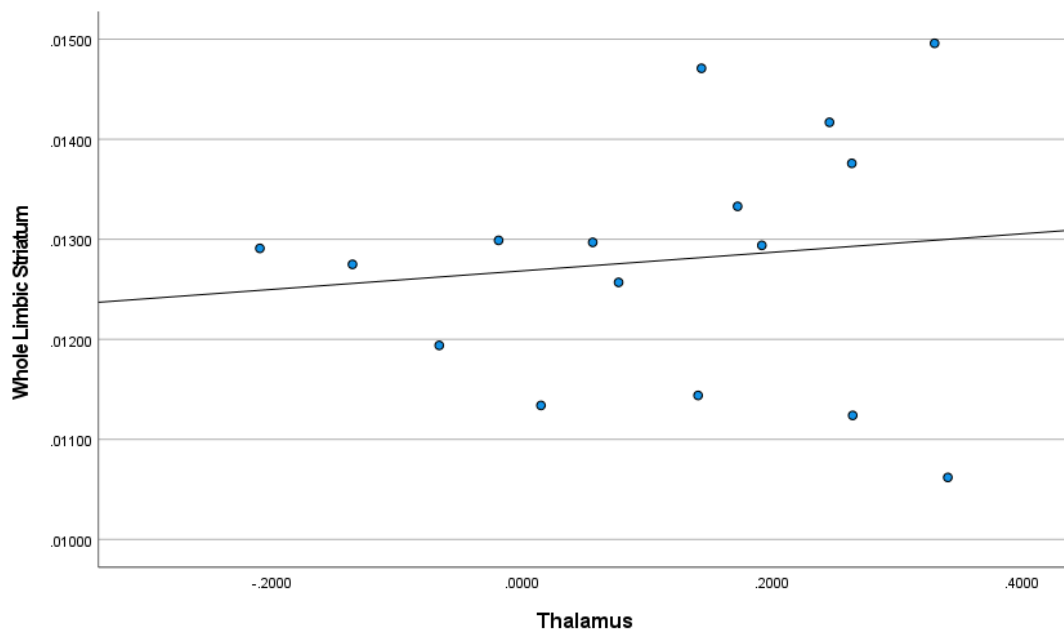
Figure 53: No significant correlations between dopamine synthesis capacity in the whole limbic striatum and BOLD response in the thalamus (during the 2-Back>1-Back contrast) in healthy controls

3.5. Discussion

There were three main findings in this study. First, there was less activation in the hippocampus and thalamus in first-episode patients than in controls during the working memory task. Secondly, there was no significant difference between the groups in dopamine synthesis capacity (DSC). In the exploratory post-hoc when patients were compared to healthy controls in whole brain analysis and where nuisance regressors encoding age, gender and 2-back performance were included at the second level, in the 2-Back>1-Back contrast there were no suprathreshold clusters. Finally, there were no significant correlations between BOLD response to the N-Back working memory task and DSC in either group or between the groups.

In the current study, there was a significant difference between first episode psychosis patients and controls in thalamus and hippocampus, but not in the dorsolateral prefrontal cortex in agreement with previous work [476, 547, 548] but in contrast to findings in some other studies [294, 296, 457]. As performance differences are a strong moderating variable when comparing neural correlates, in the current study, the analysis was restricted to data acquired during correct responses. Therefore, group differences during the performance on 2-back task are unlikely to be related to task performance and are related to differences in the neural response during the task between groups. When the Bonferroni correction was applied, only the hippocampus ROI survived the correction, whereby ROI Activation was greater in healthy controls than FEP patients when 2-Back>1-Back conditions were contrasted (height threshold p = 0.001).

When age, gender and 2-back performance were added as regressors of no interest, there were no suprathreshold clusters in healthy controls compared to patients in the hippocampus, thalamus and DLPFC small volume corrected analysis, compared to the original analysis. This suggests that there may be an impact of the covariates on the hippocampus and thalamus, but

this might be due to the smaller sample size compared with chapter 2 which did still find a significant difference when age, gender and performance were added as regressors of no interest. My exploratory findings therefore indicate that age, gender and 2-back might contribute to my findings of group differences in activation. Future research could explore the impact of age, gender, and 2-back performance on increased activation in the insula further by controlling more rigorously for performance.

There were no group differences in dopamine synthesis capacity. This is not consistent with previous reports in first episode samples [136, 145, 146, 549]. This might be due to a type II error. However, my sample size is similar to other studies that have detected a difference. Alternatively, a sub-group of patients characterised by non-response to antipsychotic treatment has been found to have relatively unaltered dopamine synthesis capacity [550, 551]. Thus, one potential explanation for my findings could be the inclusion of patients with non-responsive psychosis in the current study.

I found no significant relationship between activation in the dorsolateral prefrontal cortex, hippocampus or thalamus during working memory performance and striatal dopamine synthesis capacity. This is not consistent with my hypothesis or studies in chronic patient samples using other cognitive tasks [326], or in at-risk samples using a working memory task [139]. One of the main differences between the current study, and Fusar-Poli et al. [139] and Meyer-Lindenberg et al. [326], was the significant differences in dopamine levels between the groups in the previous studies. Thus, the difference between my findings and these previous ones could reflect the possibility that, by chance, patients with a non-dopaminergic form of psychotic illness [552] were included in my sample.

3.5.1. Limitations

The analysis was restricted to images associated with correct responses and task performance was used as a covariate, therefore it is likely that the results may reflect a true neurophysiological difference, but there are other ways of controlling for performance differences. One could also argue that this paper might have been limited by the choice of working memory task, as it might be "nonmonotonic" [553]. The usual response of the DLPFC to parametric variations in working memory load is an "inverted-U" [436, 554]. As the limited number of steps or loads with N-Back is 3 (or 4 if the 3-back condition is used), so this might restrict the ability of the task to show an inverted-U. Future work could select a different task that taps working memory (e.g., self-ordered working memory task as used by [553].

It has been suggested that the discrepancies in the literature could be related to task requirements or poor task performance in schizophrenia [436, 555]. Many have argued that task-related activation patterns are a non-linear, inverted U-shaped function that relates the fMRI signal to working memory load and is shifted to the left in patients with schizophrenia. It is also important to recognise that the first episode of psychosis cohort may include patients with affective psychoses as well as schizophrenia and that a diagnosis can change over the first few years of illness [556]. Therefore, my findings may not be specific to schizophrenia and further follow-up is required to determine the final diagnoses in the sample and if my findings are specific to schizophrenia.

3.5.2. Implications for understanding psychotic disorders

My finding that the neural basis of working memory dysfunction is not related to striatal dopamine function implies that this neurobiology of working memory dysfunction does not require striatal dopamine alterations in first episode psychosis patients. It could still involve

dopamine in the hippocampus or thalamus or be linked with dopamine in the prefrontal cortex, which was not measured in this paper.

3.5.3. Conclusion

In conclusion, this was the first study to look at the BOLD response from a working memory task and its relationship with striatal dopamine in first-episode patients with schizophrenia. There was an altered BOLD response between patients and controls in the hippocampus and thalamus, but not in the dorsolateral prefrontal cortex. There was no difference in dopamine levels between the groups and no relationship between BOLD response during the N-Back working memory task and dopamine synthesis capacity. Altered neural activation does not require striatal dopamine dysfunction.

Table 20: CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	N/A
	1- Back	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	151
Introduction			
Background and	2a	Scientific background and explanation of rationale	152
objectives	2-	Specific objectives or hypotheses	
	Back		152
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	155

	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	N/A
Participants	4a	Eligibility criteria for participants	155
	4b	Settings and locations where the data were collected	155
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	155
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	155
	6b	Any changes to trial outcomes after the trial commenced, with reasons	N/A
Sample size	7a	How sample size was determined	N/A
	7b	When applicable, explanation of any interim analyses and stopping guidelines	N/A
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	N/A
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	N/A
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	N/A

Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	N/A
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	N/A
	11-Back	If relevant, description of the similarity of interventions	N/A
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	157
	12-Back	Methods for additional analyses, such as subgroup analyses and adjusted analyses	157

Results

Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	160
	13b	For each group, losses, and exclusions after randomisation, together with reasons	160
Recruitment	14a	Dates defining the periods of recruitment and follow-up	N/A

	14b	Why the trial ended or was stopped	N/A
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	161
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	161
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	163
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	N/A
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	173
Harms	19	All-important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	N/A

Discussion

Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	181
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	181
Interpretation	22	Interpretation consistent with results, balancing benefits, and harms, and considering other relevant evidence	182

Other information

Registration	23	Registration number and name of trial registry	N/A
Protocol	24	Where the full trial protocol can be accessed, if available	N/A
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	N/A

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items.

If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological

treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up-to-date references relevant to this checklist,

see www.consort-statement.org.

4. The effects of antipsychotic treatment on brain function during a cognitive task: a prospective fMRI study in first episode psychosis

4.1. Abstract

Aim: To explore whether antipsychotic treatments affect neural response during the N-Back working memory task and whether a change in neural response linked to clinical change.

Method: 16 patients with first-episode psychosis (FEP) and 30 healthy control (HC) volunteers were included in the study. Participants were studied using functional magnetic resonance imaging whilst performing the N-Back working memory task. Blood oxygen level-dependent (BOLD) response, task performance, and the Positive and Negative Syndrome Scale (PANSS) were measured.

Results: There was hypoactivation in the DLPFC, hippocampus and thalamus in patients compared to healthy controls at baseline. With treatment, there was no significant change in activation from baseline to follow-up nor were there any significant correlations between a change in negative symptoms and a change in BOLD response during a higher working memory load.

Discussion: My findings indicate that treatment does not affect neural response during the N-Back working memory task and symptomatic improvement is not associated with altered cortical brain function in psychosis. These findings indicate that antipsychotic treatments do not have a significant effect on the neural mechanisms underlying working memory impairment in psychosis.

4.2. Introduction

WM tasks activate a network of frontotemporal brain regions that include the dorsolateral prefrontal cortex (DLPFC), thalamus and hippocampus [318, 347, 350, 351, 407, 458] (see [318] for a meta-analysis); regions which are also affected in psychosis. Compared to healthy controls, hypoactivation has been reported in the dorsolateral prefrontal cortex (DLPFC) in first-episode patients with schizophrenia [294, 299] thalamus [294, 413, 496-498] and hippocampus [499] in first episode patients compared to healthy controls in some, but not all studies [457] (see Figure 8 and 1.6.3.4 – PRISMA Checklist). This difference might be due to the inclusion of medicated patients in some studies, as antipsychotic treatment has been found to affect the functional MRI signal [277, 280, 500], and this is explored in the current experimental chapter. Working memory impairments have also been associated with more severe negative symptoms [299, 399] (see section 1.6.2), which suggest a shared neurobiological process underlying negative symptoms and working memory in psychotic disorders.

Antipsychotic medications, known to improve positive symptoms [251], have mixed effectiveness for negative and cognitive symptoms which are difficult to separate from other drug effects [252-254]. A change in frontotemporal activation levels with antipsychotic treatment has been reported by a number of studies in first episode patients [293, 294, 296, 298], but not all [291]. In patients with chronic schizophrenia, this activation has been linked with an improvement in symptoms and accuracy on a verbal working memory task [298]. In medication-naïve first episode patients [292] using a modified Sternberg working memory task, DLPFC activation deficits were unaffected by ten weeks of antipsychotic treatment. Patients who did not have symptomatic improvement ("non-responders") continued to have abnormal left DLPFC function compared to "responders" and healthy controls [292].

The results of these two studies, one in chronic patients and one in first-episode patients, contradict each other, in addition to using different working memory tasks. There are clear gaps in the first episode psychosis literature that this paper hopes to address. Firstly, the current study seeks to replicate the previous N-Back working memory findings in chronic patients but in first-episode patients with treatment. It looks to provide evidence of whether working memory deficits in FEP can be reduced by antipsychotic medication and whether this can be measured more accurately by the N-Back working memory task instead of the Sternberg WM task as the previous study in FEP did not detect any improvement with treatment. Finally, it wanted to replicate the evidence from chronic patients that activation was linked with symptom reduction.

In view of this, my first hypothesis was that antipsychotic treatments affect neural response during the N-Back working memory task. Secondly, I hypothesised that a change in neural response would be linked to clinical change.

4.3. Methods

4.3.1. Population and Sample

The East of England-Cambridge East NHS Research Ethics Committee approved this study. All participants provided informed written consent to participate.

4.3.1.1. Patient group (n =16)

45 patients, who presented to first episode psychosis services, were recruited into the main study. 26 were excluded for not starting medication and 3 patients were excluded from the analysis for too much head movement, leaving a total of 16 patients included in the study. Please see p.105 (section 2.3.1.1) for further details.

4.3.1.2. Healthy Control Group (n=30)

33 healthy controls were recruited. Please see p.106 (section 2.3.1.2) for further details and the consort flow chart at the end of this section.

4.3.1.3. Exclusion criteria for all participants:

Please see p. 106 (section 2.3.1.3) for details.

4.3.1.4. Treatment

All patients were assessed and scanned at baseline and again after at least 4 weeks of antipsychotic treatment. Assessments were performed on the same day as the follow-up scan and were conducted at least four weeks after the commencement of treatment. The study was naturalistic. The clinical team, in discussion with the patient, made the choice of antipsychotic medication. All doses were

within the therapeutic range defined in the Maudsley Prescribing Guidelines [557]. Use of other psychotropic medication (such as antidepressants and benzodiazepines) was permitted.

4.3.2. Clinical Measures:

As in chapter 2, the following four assessments were completed in the following order prior to the MRI scan: Positive and Negative Syndrome Scale (PANSS), National Adult Reading Test (NART), Global Assessment of Functioning (GAF) and a medication history – please see section 2.3.2 in the thesis for further details). These were completed prior to the MRI scan at baseline and repeated prior to the follow-up MRI scan.

4.3.3. fMRI scanning

4.3.3.1. Image acquisition

Please see p.107 (section 2.3.3.1) for details in chapter 2.

4.3.3.2. Image Analysis

As my hypotheses concerned the effect of antipsychotic treatment on brain function during a cognitive task, I performed paired t-tests as my primary analysis focused on the neural response before and after treatment with increased working memory load (i.e.: the 2-back relative to the 1-back condition). Data were analysed using Statistical Parametric Mapping (SPM8; Wellcome Department of Cognitive Neurology, London) running in MATLAB 8.0 (The MathWorks, Natick, Massachusetts). For details of pre-processing and first level pre-processing, please see p.108, section 2.3.3.2.

I employed a region-of-interest analysis approach – please see section 2.3.3.2 for further details. In the additional post-hoc analysis, 2-back performance was included as a nuisance covariate in the second level model as this might have varied between baseline and follow-up in patients. The exploratory post-hoc analysis where nuisance regressors encoding age, gender and 2-back performance were included to compare healthy controls to patients - Please see section 2.3.3.2 for further details.

4.3.4. Integration of fMRI, and PANSS data:

For contrasts where small-volume correction identified significant between-group differences, I extracted mean β-weights for each patient over the ROI (DLPFC (equivalent to Brodmann areas 9 and 46), hippocampus and thalamus) during the N-Back working memory task (2-Back>1-Back contrast) using MarsBar in SPM8 and correlated these with the PANSS or Marder negative symptom scores for patients in SPSS.

4.3.5. Statistical Analysis

The effect of group on demographics and clinical measures scores were tested using analysis of variance for parametric variables and the Mann-Whitney U tests for non-parametric variables after checking for the equality of variance using the Levene test, or chi-squared/Kruskal-Wallis for categorical data. A MANOVA was used to test any group differences in the behavioural N-Back data. Where the Mauchly's test indicated that the assumption of sphericity had been violated, the degrees of freedom were corrected using Huynh-Feldt estimates of sphericity ($\varepsilon < 0.75$).

One sample t-tests were used to look at the main effect of the task at baseline in patients and controls, and in patients at follow-up after treatment. A two-sample t-test was used to compare the healthy

control group with the patients with first-episode psychosis at baseline. Paired sample t-tests were used to test for the difference from baseline to follow-up in PANSS and GAF scores. To test the hypothesis that there is a reduction in activation in the following ROIs: dorsolateral prefrontal cortex, hippocampus, and thalamus, and with treatment, I performed paired-samples t-tests at a second-level analysis comparing activation during N-Back, for 2-back>1-back contrasts between the baseline and follow-up. The whole brain voxel-wise threshold was set at p<0.001, uncorrected. The ROI voxel-wise threshold was set at p < 0.001, uncorrected. As I have used an uncorrected threshold, I have unquantified control of family-wise error.

Pearson's correlations were used where the data parametric. Where the data is not parametric, Spearman's Rho was used. To test whether there was a correlation between activation and negative and cognitive symptoms, a multiple regression model was used to identify regions where the change in activity was explained by the change in PANSS (or vice versa).

Difference images were created for each participant (baseline minus follow-up) in ImCalc (i1-i2) for each of the five contrasts (detailed above) along with difference PANSS scores for each participant (again, baseline minus follow-up). Difference negative symptom scores were created from PANSS and Marder [510] scores for each participant. The following equation was used to calculate the difference scores: $d = ((X-7) - (Y-7))$, where d is the difference score, X is the baseline score (e.g., PANSS, Marder, TMT.), Y is the follow-up score and -7 is the floor removed. β-values were extracted from the significant whole brain analysis areas using predefined AAL ROIs (e.g., hippocampus). *Post hoc* one-tailed (p<0.05) correlations were carried out between the β-values and the difference in PANSS scores. Outliers in the data were identified via boxplots and removed.

4.4. Results
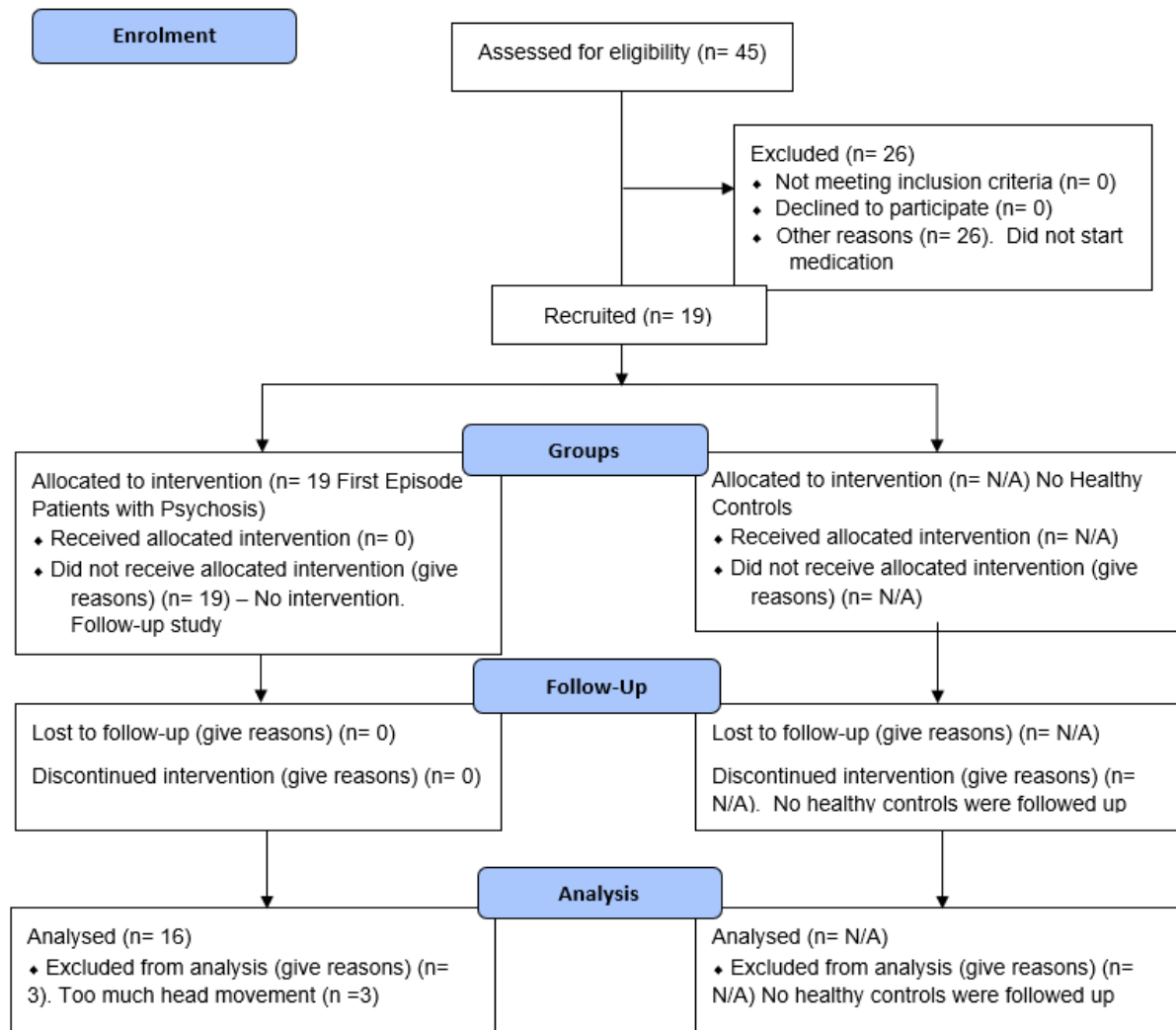
Figure 54: CONSORT 2010 Flow Diagram

4.4.1. Participants

In total sixteen patients who were included in the study and thirty healthy controls. For the attrition rate, please see the consort flowchart (Figure 54); 45 patients were initially recruited. 19 started medication and agreed to be followed up. 3 patients were removed due to excessive head movement during the fMRI paradigm. The mean (s.d.) duration of untreated psychotic illness was 22.82 (s.d. 20.3) months (see Table 21). Patients had median of 39 days of treatment when scanned at follow-up. There was a mean of 167 days between baseline and follow-up scan (166.94 (s.d. 290.17) and median of 43.0 (IQR 36.0) days when medication began relative to assessments. Patients were moderately ill with a mean [sd] negative PANSS score at baseline of 15.62 [$\pm$5.8], a negative Marder score of 16.19 [$\pm$7.2], PANSS positive (19.06 [$\pm$ 6.75]) and PANSS total (69.82 [$\pm$ 22.29]) (see Figure 55, Table 21 and Table 22).

At follow-up, there was a significant reduction in negative PANSS scores (12.25 [$\pm$6.5]), Marder negative PANSS scores (12.06 [$\pm$6.2]), PANSS Positive (11.85 [$\pm$4.54] and PANSS total scores (48.56 [$\pm$ 15.78]. A repeated measures ANOVA showed that there was no significant main effect of time, $F(1,15) = 0.348$, $p = 0.938$ from baseline to follow-up on how well patients performed on the N-Back task. The behavioural scores for the N-Back were out of a total of 12 for each level of difficulty i.e., 0–back = score out of 12, etc. There was a significant main effect of N-Back level, $F(2,30) = 6.192$, $p = 0.006$. There was no significant interaction between time and N-Back level, $F(2,30) = 0.308$, $p = 0.737$. No other significant differences between baseline and follow-up time points for performance on the N-Back task (see Figure 55 and Table 22).

4.4.2. Demographics

Table 21 – Demographics and clinical characteristics

Characteristics		Healthy Controls[+]	Patients
Sex (female: male)		8:22	5:11
Age ((mean years) [SD])		24.68 [3.88]	25.06 [4.09] years
NART Score		36.38 [8.02]	30.38 [7.67]
Handedness (% R)		92%	86.4%
Duration of untreated psychosis (months)[1,2]		N/A	22.82 [20.33]
Medication status at entry to study		N/A	7 (TN), 4 (MT), 5 (TF)
Diagnosis		N/A	9 (FEP); 7 (BPAD)
Antipsychotic treatment initiated	Amisulpride	N/A	n = 5
	Aripiprazole	N/A	n = 4*
	Lurasidone	N/A	n = 1
	Olanzapine	N/A	n = 2*
	Paliperidone	N/A	n = 1
	Quetiapine	N/A	n = 2
	Risperidone	N/A	n = 2

TN – Treatment naïve; MT – minimally treated; TF – treatment free; FEP = first episode psychosis;

BPAD – bipolar affective disorder. *- one patient was taking both aripiprazole and olanzapine. [+] -

healthy controls are part of an overlapping sample

Table 22: Symptom Scores, N-Back correct trials (out of 12) and trail-making task (Mean [SD]) baseline and follow up general symptoms.

	Baseline	Follow-Up	Paired t-test results
GAF	48.20 [14.9]	66.60 [18.1]	t(14) = 3.924, p = 0.002
PANSS Positive	19.06 [6.75]	11.85 [4.54]	t(15) = 4.04, p = 0.001
PANSS Negative	15.62 [5.82]	12.25 [6.47]	t(15) = 2.222, p = 0.042
Marder Negative	16.19 [7.21]	12.06 [6.22]	t(15) = 2.948, p = 0.010
PANSS Total	69.82 [22.29]	48.56 [15.78]	t(15) = 4.162, p = 0.001
0-Back	11.69 [0.602]	11.69 [1.014]	t(15) = p<0.001, p = 1.000
1-Back	11.19 [1.223]	11.69 [0.602]	t(15) = 1.576, p = 0.136
2-Back	10.94 [1.482]	10.69 [2.152]	t(15) = 0.417, p = 0.682

GAF – Global Assessment of Functioning; PANSS – Positive and Negative Syndrome Scale; 0-Back, 1-Back, and 2-Back – 3 Levels of N-Back Working Memory Task.
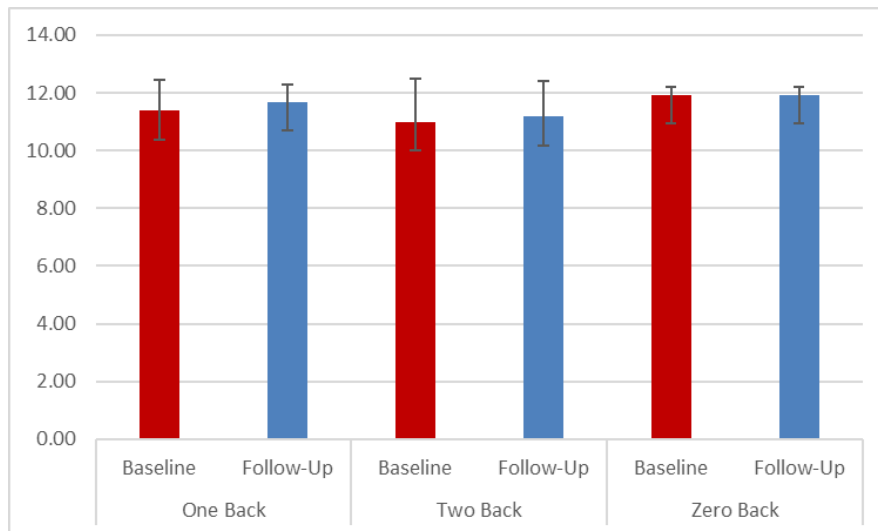
Figure 55: Mean N-Back behavioural scores out of 12 for patients with FEP at baseline and at follow-up time points.

4.4.3. fMRI Results

4.4.3.1. Formal Test of Head Movement

The paired sample t-test results show that there was no significant effect of head movement between the baseline and follow-up t(3) = 0.582, p = 0.602, see Figure 56)
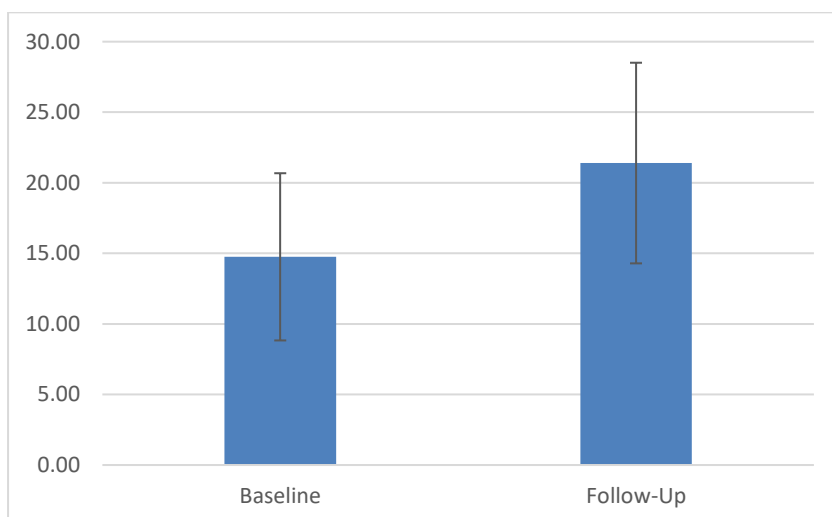
Figure 56: Mean Head Movement for Patients and Healthy Controls (in millimetres)

4.4.3.2. Main Effect of Task (baseline results)

During the task at baseline, there was activation in the middle and superior frontal gyri in patients and controls. In patients at follow-up, there was activation in the SFG and inferior parietal lobule (see Table 23 and Figure 57).

Table 23: **Main effect of task: Foci of Brain Activation during the N-Back Task in healthy controls, baseline patients and follow-up patients (height threshold p < 0.001 unc, cluster level P < 0.05 FWE). Contrast - 2-Back>1-Back**

	Region	Left or Right	MNI Coordinates			Number of Voxels	z-scores	Cluster Level pFWE-corrected
			X	y	z			
Patients Baseline	Supramarginal gyrus	R	48	-36	44	4961	5.43	p<0.001
	Superior Frontal Gyrus	L	-20	0	50	2658	5.00	p<0.001
	Middle Frontal Gyrus	R	32	4	52	1174	4.93	p<0.001
	Middle Frontal Gyrus	R	42	30	38	505	4.18	0.010
Controls Baseline	Superior Frontal Gyrus	R	22	-2	54	28905	6.18	p<0.001
	Middle Frontal Gyrus	L	-38	52	16	515	4.28	0.031
Patients Follow-Up	Inferior Parietal Lobule	R	42	-56	42	910	4.31	0.003
	Superior Frontal Gyrus	R	30	18	56	644	4.07	0.012
	Inferior Parietal Lobule	L	-30	-54	42	772	3.79	0.006
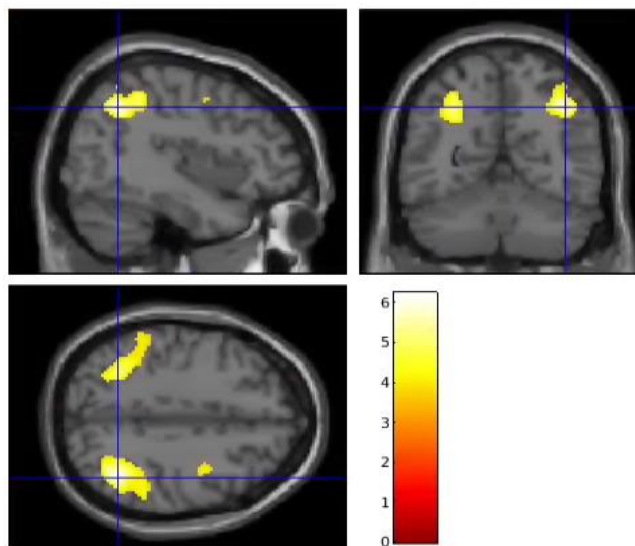
Figure 57: Showing regions significantly activated during the 2-Back>1-Back contrast in patients at the follow-up timepoint (cluster level pFWE corrected<0.05), whole brain analysis. The colour bar indicates the t value.

In the whole brain analysis, there was hypoactivation in patients compared to healthy controls during a high working memory load than a lower working memory load (see Table 24 and Figure 58) in a whole-brain analysis in superior frontal gyrus (p = 0.034), and in the small volume corrected regions of interest (see Table 25 and Figure 59, Figure 60, and Figure 61). These healthy controls were from an overlapping sample (see previous experimental chapters). None of the ROIs survived the Bonferroni correction (see Table 25).

Table 24: Comparison with healthy controls > first episode patients at baseline for 2-Back>1-Back contrast (height threshold p = 0.001 unc), whole brain analysis.

Region	Left or Right	MNI Coordinates			Number of Voxels	z-scores	Cluster Level FWE
		x	y	z			
Superior Frontal Gyrus	R	24	0	58	476	4.01	0.034
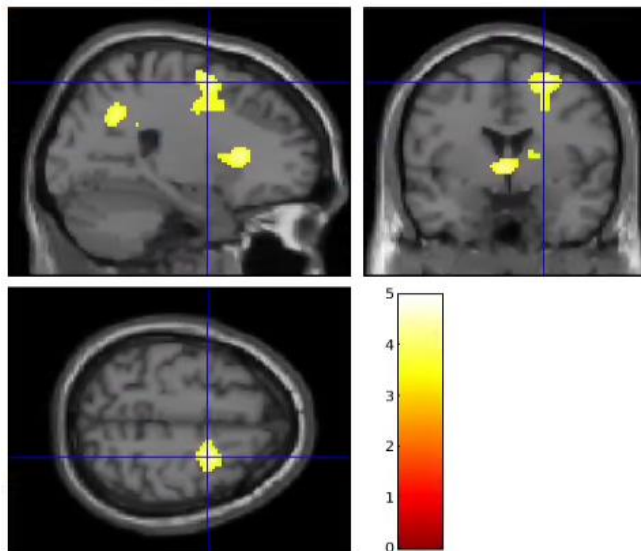
Figure 58: Showing regions significantly activated during the 2-Back>1-Back contrast in patients at the baseline timepoint compared to healthy controls, (cluster level pFWE corrected<0.05), whole brain analysis. The colour bar indicates the t value.

Table 25: Comparison with healthy controls > first episode patients at baseline for 2-Back>1-Back contrast (height threshold p = 0.001 unc), Small Volume Corrected (s.v.c) Regions of Interest (ROI) analysis.

ROI	Region	Left or Right	MNI Coordinates			Number of Voxels	z-scores	Peak Level pFWE-corrected	FWE error corrected with additional Bonferroni Correction (p<0.0167)
			x	y	z				
DLPFC	Precentral Gyrus	Left	-58	6	30	18	3.77	0.043	N/S
Hippocampus	Hippocampus	Right	22	-34	10	4	3.29	0.059*	N/S
Thalamus	Thalamus	Right	20	-30	12	3	3.37	0.036	N/S
Thalamus	Thalamus	Right	8	-26	12	10	3.22	0.055*	N/S

N/S = Not Significant; *trend-level significance
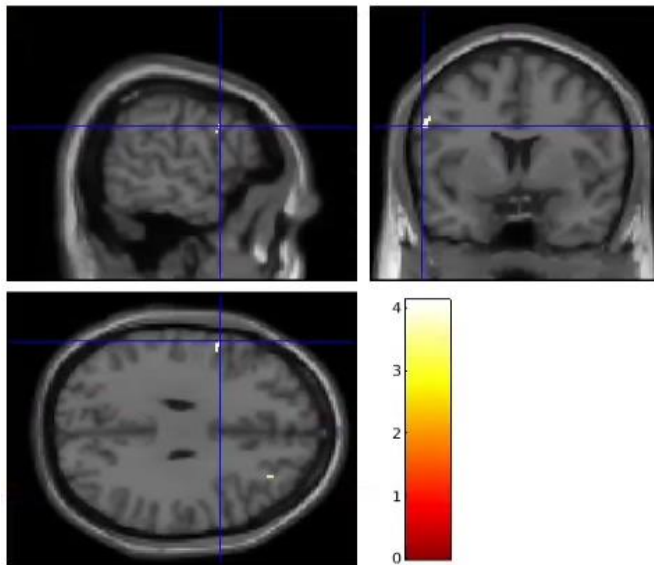
Figure 59: Showing regions significantly activated during the 2-Back>1-Back contrast in patients at the baseline timepoint compared to healthy controls. For hypothesis-led ROI analyses, statistical maps were initially thresholded at an uncorrected height threshold of p<0.001, followed is family-wise error correction via small volume correction (SVC; pFWE<0.05) using DLPFC ROI. The peak is shown by the cross hair. The colour bar indicates the t value.
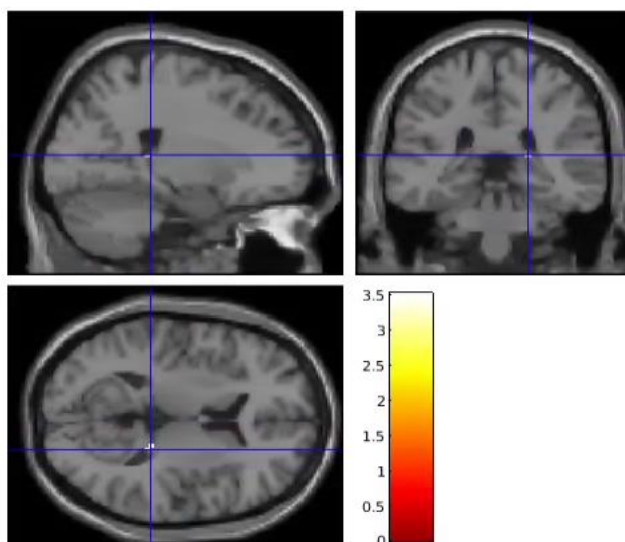
Figure 60: Showing regions significantly activated during the 2-Back>1-Back contrast in patients at the baseline timepoint compared to healthy controls. For hypothesis-led ROI analyses,

statistical maps were initially thresholded at an uncorrected height threshold of p<0.001, followed is family-wise error correction via small volume correction (SVC; pFWE<0.05) using hippocampus ROI. The peak is shown by the cross hair. The colour bar indicates the t value.
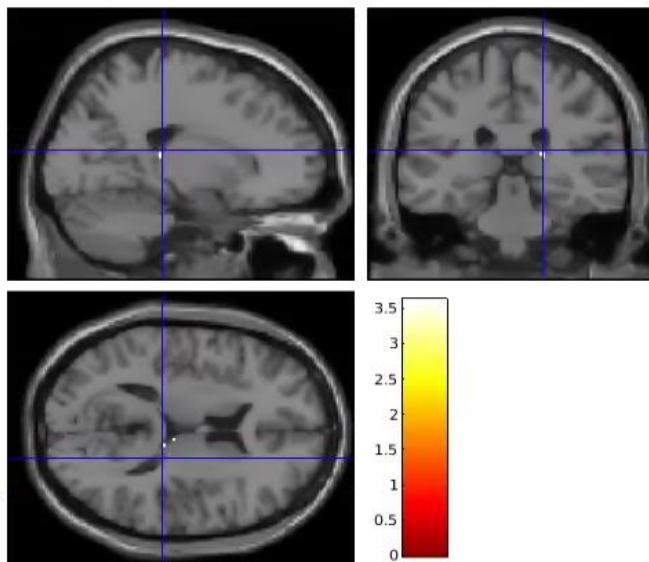
Figure 61: Showing regions significantly activated during the 2-Back>1-Back contrast in patients at the baseline timepoint compared to healthy controls. For hypothesis-led ROI analyses, statistical maps were initially thresholded at an uncorrected height threshold of p<0.001, followed is family-wise error correction via small volume correction (SVC; pFWE<0.05) using thalamus ROI. The peak is shown by the cross hair. The colour bar indicates the t value.

4.4.3.3. Baseline Versus Follow-Up Patients:

In my primary analysis (2-back>1-back), there were no suprathreshold clusters when baseline was compared to follow-up (baseline>follow-up) patients in the whole brain analysis. There were no significant cluster-level clusters in the whole brain analysis when follow-up was compared to

baseline (follow-up>baseline; see Table 26 and Figure 62) and there were no suprathreshold

clusters in the region of interest (ROI) analyses.

Table 26: Follow-up first episode patients > baseline patients, 2-Back>1-Back contrast (height threshold p = 0.001 unc), whole brain analysis.

Region	Left or Right	MNI Coordinates			Number of Voxels	z-scores	Cluster Level FWE
		x	y	z			
Precentral Gyrus	Right	54	-8	30	35	3.63	0.735
Inferior Frontal Gyrus	Left	-54	8	16	21	3.47	0.813
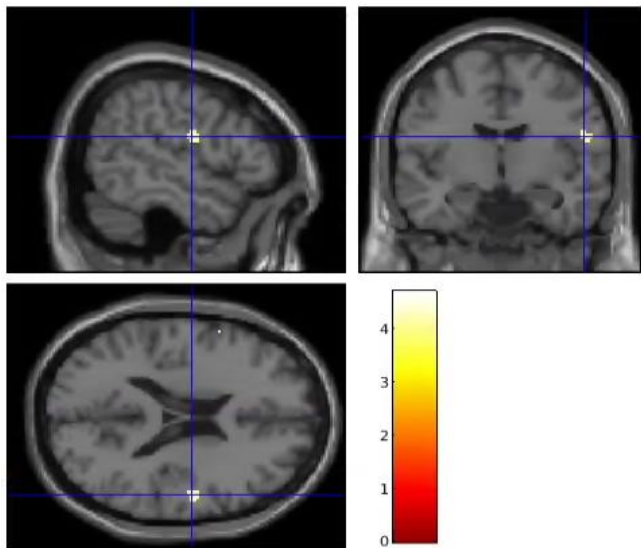
Figure 62: Showing regions activated during the 2-Back>1-Back contrast in patients at the baseline timepoint compared to patients at the follow-up timepoint, whole brain analysis

(cluster level threshold pFWE corrected < 0.05). The peak is shown by the cross hair. The colour

bar indicates the t value.

4.4.3.4. Nuisance Covariates - Age, Gender, and 2-Back Performance

There was no significant grey matter activation in healthy controls compared to patients at baseline

in the whole brain analysis, but there was significant activation in the hippocampus and thalamus

ROIs (see Table 27, Figure 63 and Figure 64). The hippocampus and thalamus ROIs survived

familywise error correction (pFWE<0.05), only the thalamus reached the more conservative

threshold based on the Bonferroni correction for the number of independent ROIs used (i.e.,

pFWE<0.0167). There were no suprathreshold clusters in patients at baseline when 2-back

performance was a regressor of no interest compared to the follow-up time point (baseline>follow-

up) in the whole brain analysis. When the follow-up time point was compared to baseline patients,

there was no significant activations (follow-up>baseline; see Table 28 and Figure 65).

Table 27: Region of Interest (ROI) Increased Activation during the N-Back Task in healthy controls compared to patients at baseline for 2-Back>1-Back Contrast in the hippocampus and thalamus (s.v.c) when age, gender and 2-back performance were covaried.

ROI	Region	Left or Right	MNI Coordinates			No. of Voxels	z-scores	Cluster Level (FWE correction)	FWE error corrected with additional Bonferroni Correction (p<0.0167)
			x	y	z				
Hippocampus	Hippocampus	Right	22	-34	10	10	3.60	0.023	N/S
Thalamus	Thalamus	Right	20	-30	12	18	3.71	0.013	Significant

N/S = not significant
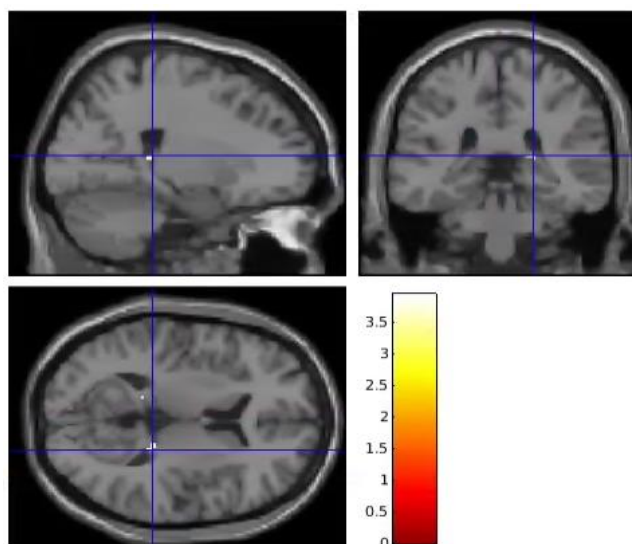
Figure 63: Statistical maps illustrating regions of significantly increased activation during the N-Back Task in healthy controls compared to patients at baseline for the 2-Back>1-Back Contrast.

For hypothesis-led ROI analyses, statistical maps were initially thresholded at an uncorrected height threshold of p<0.001, followed is family-wise error correction via small volume correction (SVC; pFWE<0.05) using hippocampus ROI, where nuisance regressors encoding age, gender and 2-back performance were included. The peak is shown by the cross hair. The colour bar indicates the t value.
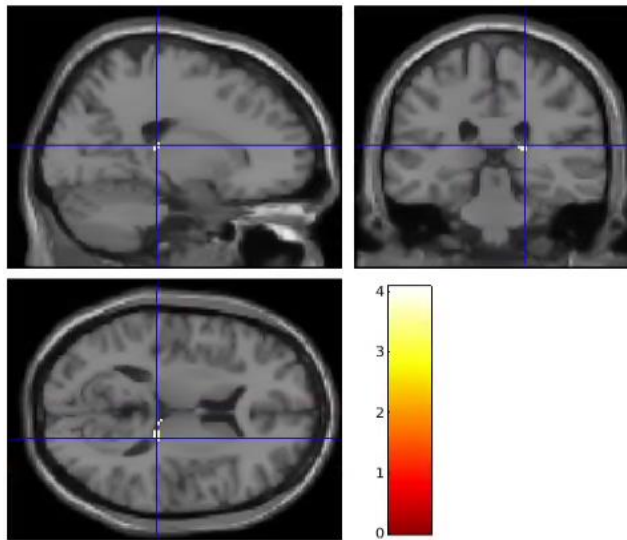
Figure 64: Statistical maps illustrating regions of significantly increased activation during the N-Back Task in healthy controls compared to patients at baseline for the 2-Back>1-Back Contrast . For hypothesis-led ROI analyses, statistical maps were initially thresholded at an uncorrected height threshold of p<0.001, followed is family-wise error correction via small volume correction (SVC; pFWE<0.05) using thalamus ROI, where nuisance regressors encoding age, gender and 2-back performance were included. The peak is shown by the cross hair. The colour bar indicates the t value.

Table 28: Whole brain analysis - Foci of Brain Activation during the 2-Back>1-Back contrast in patients at baseline compared to follow-up (baseline<follow-up) when age, gender and 2-back performance were covaried.

Region	Left or Right	MNI Coordinates			No. of Voxels	z-scores	Cluster Level (FWE correction)
		x	y	z			
Rolandic Operculum	Right	54	-8	20	29	4.59	0.770
Precentral Gyrus	Left	-52	6	16	21	3.52	0.815
Insula	Right	38	6	18	6	3.44	0.904
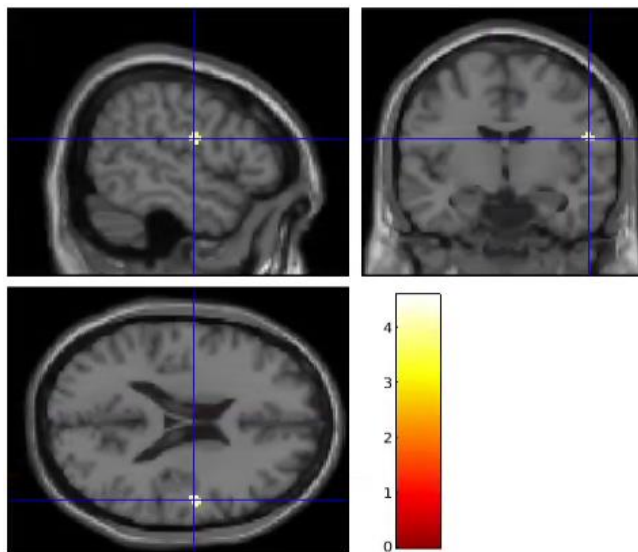
Figure 65: This shows the foci of brain activation during the 2-Back>1-Back contrast in patients at baseline compared to follow-up, cluster level pFWE corrected<0.05), whole brain analysis. when age, gender and 2-back performance were covaried. The peak is shown by the cross hair. The colour bar indicates the t value.

4.4.3.5. Neural Response and Clinical Change:

To explore the secondary hypothesis that alterations in neural responses might be linked to clinical change (as measured by the PANSS), a multiple regression model was used. There were no significant correlations from the multiple regression model for the 2-Back>1-Back contrast in the whole brain or for any regions where the change in activity was explained by the change in positive PANSS, negative PANSS (see Figure 66, Figure 67 and Figure 68), total PANSS or Marder Negative Sub-Scale (see Figure 69, Figure 70 and Figure 71). Furthermore, there were no correlations between the change in BOLD in the following ROIs: hippocampus, thalamus or middle frontal gyrus, and changes in the PANSS positive score, PANSS total score or GAF scores.
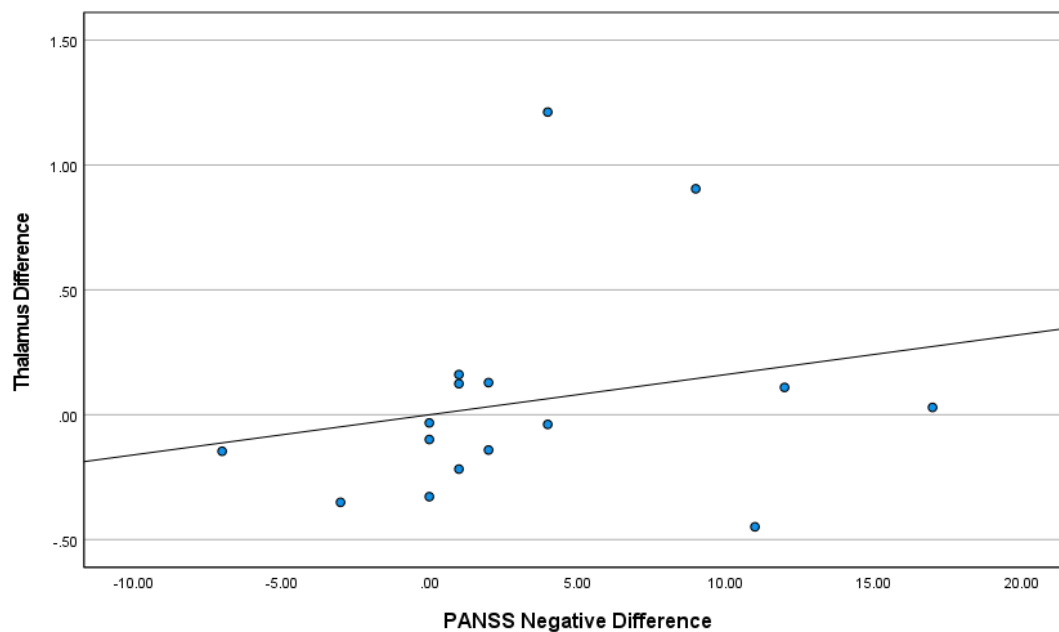
Figure 66: No significant correlations for the 2-Back>1-Back contrast in the thalamus where the change in activity was explained by the change in negative PANSS (i.e., baseline minus follow-up e.g. PANSS negative baseline score – PANSS negative follow-up score = PANSS negative difference)
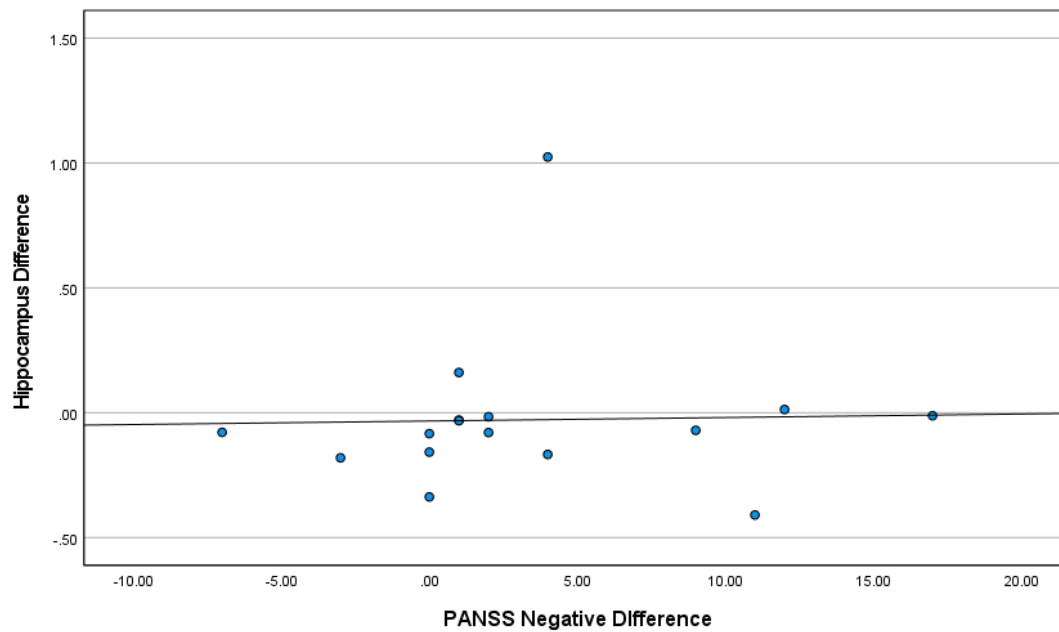
Figure 67: No significant correlations for the 2-Back>1-Back contrast in the hippocampus where the change in activity was explained by the change in negative PANSS
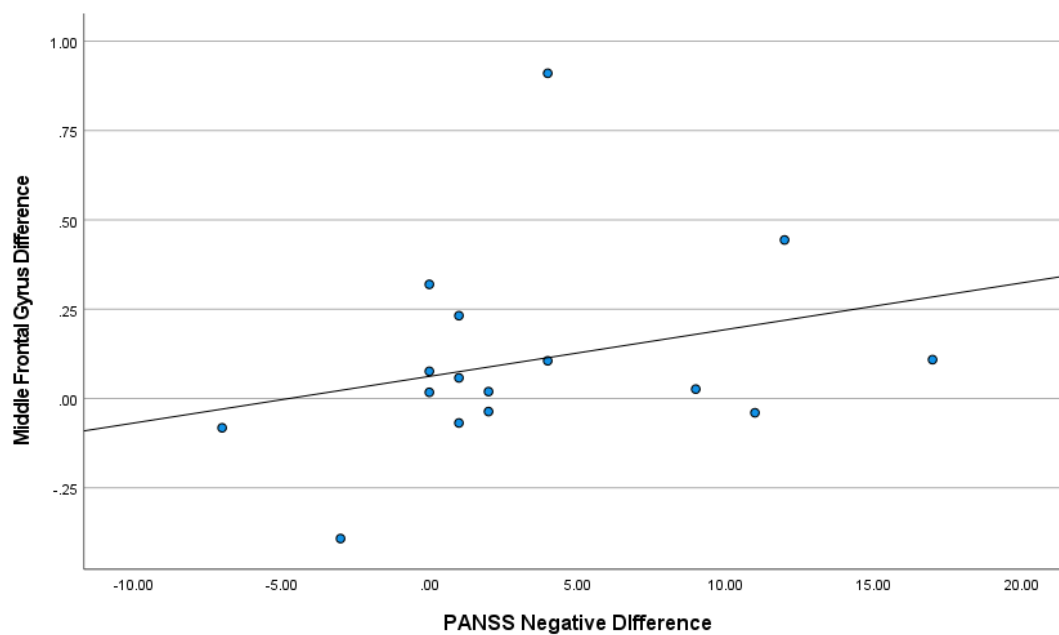
Figure 68: No significant correlations for the 2-Back>1-Back contrast in the middle frontal gyrus (MFG) where the change in activity was explained by the change in negative PANSS
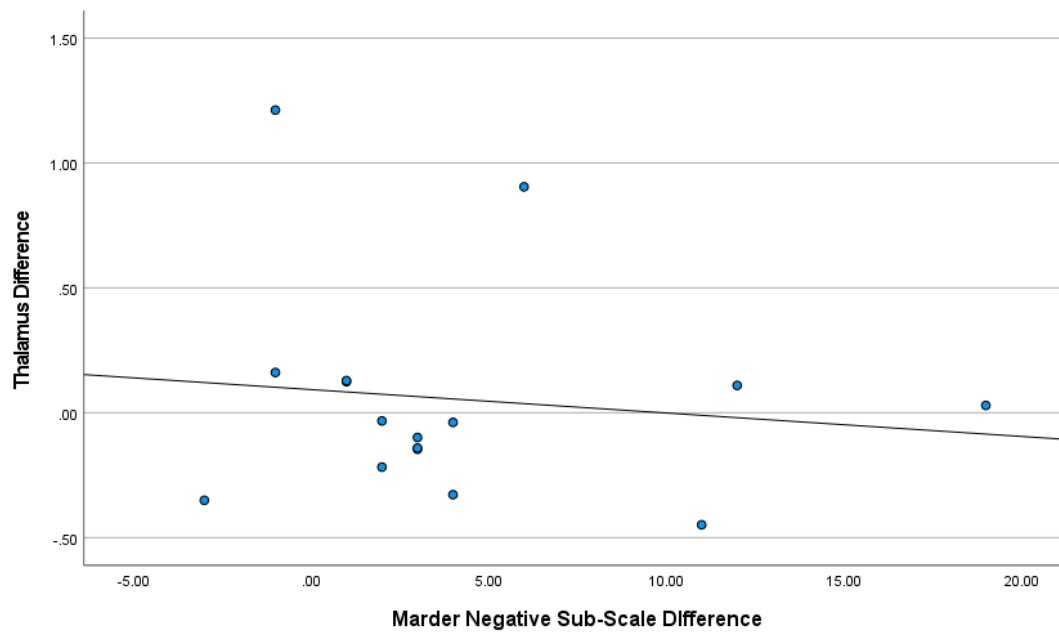
Figure 69: No significant correlations for the 2-Back>1-Back contrast in the thalamus where the change in activity was explained by the change in Marder Negative Sub-Scale
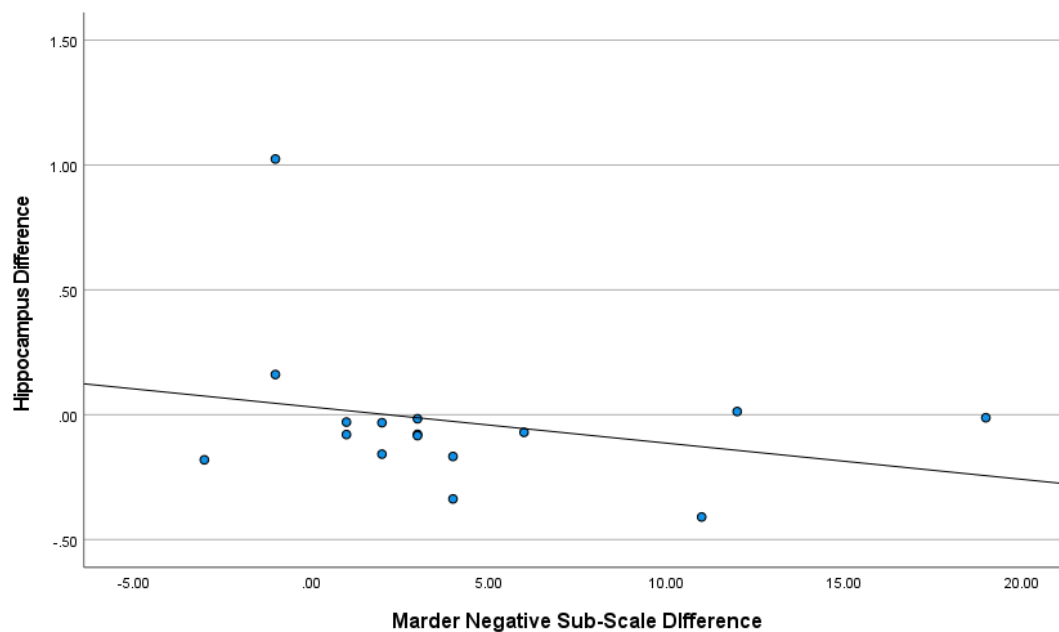
Figure 70: No significant correlations for the 2-Back>1-Back contrast in the hippocampus where the change in activity was explained by the change in Marder Negative Sub-Scale
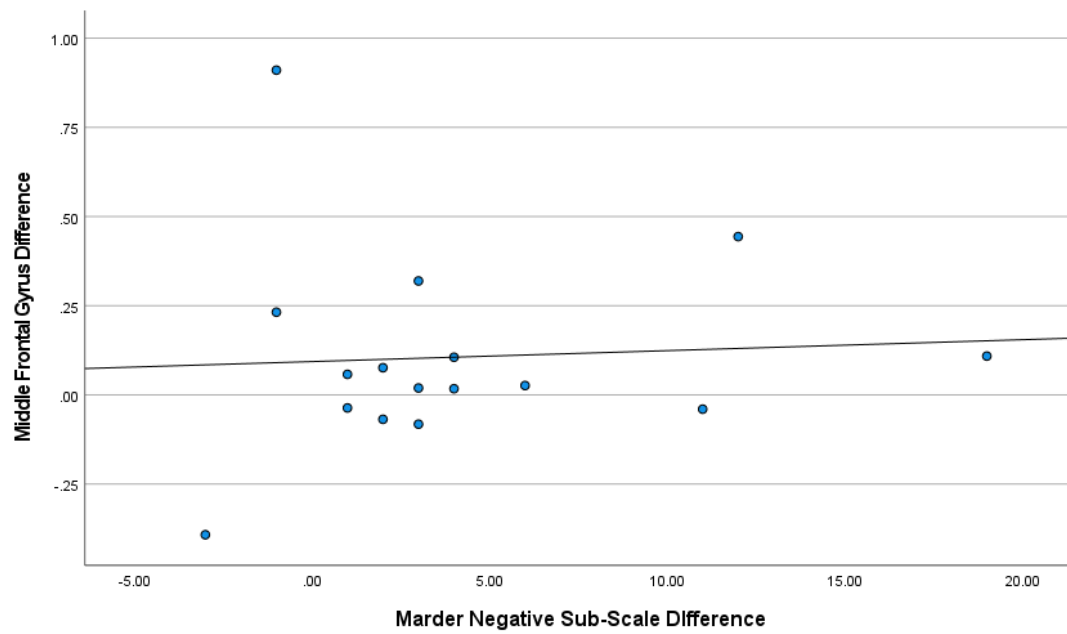
Figure 71: No significant correlations for the 2-Back>1-Back contrast in the middle frontal gyrus where the change in activity was explained by the change in Marder Negative Sub-Scale

4.5. Discussion

This study examined whether dorsolateral prefrontal cortex, thalamic and hippocampal dysfunction in patients with first-episode psychosis is altered by antipsychotic medication and whether this dysfunction is related to clinical change with antipsychotic treatment. The main findings of the current study were first there were no significant differences in activation from baseline to follow-up, therefore antipsychotic medication did not affect neural response during the N-Back working memory task. In the original analysis, DLPFC and thalamus ROI analyses had greater activation in healthy controls compared to patients at baseline, but these did not survive the Bonferroni correction. In the post-hoc exploratory ROI analysis where age, gender and 2-back performance were covaried, there was increased hippocampus activation in controls compared to patients at baseline, but this did not survive the Bonferroni correction.

In the original analysis, DLPFC and thalamus ROI analyses had greater activation in healthy controls compared to patients at baseline. While they survived familywise error correction ($pFWE<0.05$) they did not reach the more conservative threshold based on the Bonferroni correction for the number of independent ROIs used (i.e., $pFWE<0.0167$). In the post-hoc exploratory ROI analysis where age, gender and 2-back performance were nuisance covariates, there was increased hippocampus activation in controls compared to patients at baseline. Again, while they survived familywise error correction ($pFWE<0.05$), the thalamus did reach the more conservative threshold based on the Bonferroni correction for the number of independent ROIs used (i.e., $pFWE<0.0167$), whilst the hippocampus did not. In the original analysis, there were no suprathreshold clusters when patients at baseline were compared to patients at follow-up and nor was there in the post-hoc analysis (baseline>follow-up) that included nuisance covariates. There were no significant activations when follow-up patients were compared to baseline patients (follow-up>baseline) in either the original or exploratory post-hoc 2-back performance nuisance regressor analyses.

Finally, there were no significant correlations for any region where the change in activity was explained by the change in positive PANSS, negative PANSS, total PANSS or Marder Negative Sub-Scale in the higher working memory load, compared to the lower working memory load. Therefore, my second hypothesis can also be rejected, as I did not find any evidence linking a change in neural response with clinical change in patients with first-episode psychosis.

4.5.1. Change in Brain Activity with Treatment

The lack of effect of antipsychotic treatment on DLPFC, thalamus and hippocampus function in my paper is in line with findings from a follow-up study of working memory in first-episode patients [292] but not in line with other studies of working memory in first-episode patients [293, 294, 296] or chronic patients [298]. Van Veelen at al. (2011) [292] found a lack of effect of antipsychotics in first-episode patients with psychosis during a working memory task after 10 weeks of treatment with antipsychotic medication. The differences found between my paper and those by Mendrek et al (2004) [294] and Meisenzahl et al (2006) [296] come down to three main factors: i. different N-Back contrasts used; ii. Performance on the working memory task was not always controlled for, and iii. Sample size and treatment differences.

Medrek et al. (2004) [294] and Meisenzahl et al, (2006) [296] both used 2-Back versus 0-Back conditions, compared to the current paper that used 2-Back>1-Back. The 0-Back condition requires a button push for the same target letter (e.g. X), thus involving sustained attention but no actual working memory demand [558]. Comparing 2-Back with 0-Back uses other processes other than just working memory, such as attentional and executive processes as well [559]. Therefore, could be argued that it is not a comparator condition against a working memory condition. Medrek et al.

(2004) [294] did not control for performance on the N-Back, whilst Meisenzahl et al. (2006) [296]

did. Finally, Medrek et al. (2004) [294] and Meisenzahl et al. (2006) [296] had relatively small

sample sizes of n=8 and n=12 respectively. Admirably, van Veelen et al. (2011) [292] had a sample

size of n=23 in their paper, but as with the current study did not find any differences between

baseline and follow-up with antipsychotic medication until they divided their sample into

responders and non-responders. In addition to this, van Veelen et al. (2011) used the Sternberg

working memory task compared to my N-Back working memory task. Finally, one final critique

was that the patients in Medrek et al's [294] paper were all minimally treated for three days prior

to their first scan, compared to the current study where 75% of the patients were treatment-naïve

or treatment-free prior to baseline scanning.

4.5.2. Neural Response and Clinical Change

The lack of change in neural response linked to clinical change is in line with one chronic patient

study [560] but opposed to one study in first episode patients [292] and one with chronic patients.

Honey et al. (2003) found activation in the superior temporal cortex in chronic patients, but no

association with negative or cognitive symptoms [560]. Van Veelen et al (2011) [292] demonstrated

that a reduction in symptoms had an effect on activation levels with medication. Wolf et al. (2007)

[298] using a verbal working memory task, found a bilateral increase in frontotemporal function

was associated with symptom reduction with treatment. No other studies have looked at

hippocampal or thalamic activation change with treatment. A few studies in first-episode patients

and N-Back task, have found an association between negative symptoms and frontal activation

[461] or DLPFC dysfunction and the severity of negative symptoms and disorganisation [299].

Perlstein et al. (2001) showed that greater DLPFC activation was associated with disorganised

symptoms in medication patients with schizophrenia [427]. In a follow-up study in 11 first episode

patients using a working memory task (Overcome Prepotency) Snitz et al. (2005) [293], they found

that after 4 weeks of antipsychotic treatment, there was no difference in activation in the DLPFC nor were there any significant correlations between activation and symptoms. Furthermore, there were no significant correlations between change in symptom ratings and change in dorsolateral prefrontal cortex activation from baseline to 4 weeks.

4.5.3. Limitations

In the current study, the sample size was relatively small. Another possible limitation was the possibility of practice effects, but Van Veelen et al (2011) demonstrated that practice effects did not impact on their results [292]. 2-back performance was a regressor of no interest in the exploratory post-hoc analysis. These small differences in the original compared with the nuisance aggressor analysis might have been due to the smaller sample size in this chapter. Future research might want to test further with a larger sample and controlling more rigorously for age, gender, and performance. In healthy controls, it has been demonstrated that continuous practice may have an effect on the activation of the PFC during the N-Back task [561]. This paper was a naturalistic study and the clinical team, in discussion with the patient, made the choice of antipsychotic medication. Therefore, the absence of an effect of treatment on neural activation should be interpreted with caution possibly due to the different antipsychotics that the patients were on.

It is not possible to separate specific antipsychotic treatment effects on negative symptoms from non-specific changes in negative symptoms related to the natural course of the illness. Including a placebo-treated control group of schizophrenia patients, who for ethical reasons is not feasible, could help disentangle these factors.

4.6. Conclusion

In conclusion, hypofunction in the DLPFC, hippocampus and thalamus were present in first-episode treatment-free patients with schizophrenia but did not change with treatment. In addition, there was no relationship between neural response during the working memory task and symptomatic change in patients. These findings suggest that treatment does not target neural mechanisms underlying working memory impairment. Cognitive deficits are linked to important outcomes such as social functioning [562] and independent living [563]. A greater understanding of the effects of antipsychotic medication on these systems is crucial to improving the treatment of schizophrenia, especially in the treatment of cognitive symptoms.

Table 29: CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	N/A
	1-	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	188
	Back		
Introduction			
Background and	2a	Scientific background and explanation of rationale	189
objectives	2-	Specific objectives or hypotheses	
	Back		189
Methods			

Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	191
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	191
Participants	4a	Eligibility criteria for participants	191
	4b	Settings and locations where the data were collected	192
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	192
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	192
	6b	Any changes to trial outcomes after the trial commenced, with reasons	N/A
Sample size	7a	How sample size was determined	N/A
	7b	When applicable, explanation of any interim analyses and stopping guidelines	N/A
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	N/A
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	N/A

Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	N/A
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	N/A
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	N/A
	11-Back	If relevant, description of the similarity of interventions	N/A
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	193
	12-Back	Methods for additional analyses, such as subgroup analyses and adjusted analyses	193
Results			
	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	195

Participant flow (a diagram is strongly recommended)	13b	For each group, losses, and exclusions after randomisation, together with reasons	195
Recruitment	14a	Dates defining the periods of recruitment and follow-up	196
	14b	Why the trial ended or was stopped	N/A
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	196
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	199
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	199
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	N/A
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	204
Harms	19	All-important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	N/A

Discussion

Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	218
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	219
Interpretation	22	Interpretation consistent with results, balancing benefits, and harms, and considering other relevant evidence	219

Other information

Registration	23	Registration number and name of trial registry	N/A
Protocol	24	Where the full trial protocol can be accessed, if available	N/A
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items.

If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological

treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up-to-date references relevant to this checklist,

see www.consort-statement.org.

5. **Thesis Discussion**

The research in this PhD focused on the investigation of first-episode patients with psychosis. In the first part of the thesis, I used functional MRI and PET imaging to investigate two hypotheses concerning the pathophysiology of schizophrenia: (1) whether dorsolateral prefrontal cortex (DLPFC), hippocampal and thalamic dysfunction was related to negative symptoms and (2) whether this dysfunction was related to striatal dopamine function. In the second part of the thesis, I explored the short-term effects of antipsychotic treatment, principally if (3) treatment affected working memory neural response and if this was related to clinical change. I will now consider the hypotheses I aimed to test and whether the findings of the studies support them, before considering the implications for understanding schizophrenia, the limitations of the studies and future directions.

Hypothesis 1:

There would be less activation in the dorsolateral prefrontal cortex (DLPFC), thalamus and hippocampus during high working memory load relative to lower load in patients than controls.

I tested this hypothesis in the study presented in chapter 2. The results were patients (FEP) displayed blunted load-related (2-Back>1-Back) responses, relative to HCs, in the thalamus ($p = 0.018$) and hippocampus ($p = 0.023$). This partially supported hypothesis 1.

Hypothesis 2:

Lower activation, as measured by the BOLD response during performance on the N-Back working memory task, would be directly associated with greater severity of negative symptoms in patients.

I tested this hypothesis in the study presented in chapter 2. The results were that PANSS negative symptom scores did not correlate with this reduced BOLD response. This did not support hypothesis 2.

Hypothesis 3:

The BOLD response in the DLPFC, hippocampus and thalamus would be associated with greater striatal dopamine function.

I tested this hypothesis in the study presented in chapter 3. The results were there were no significant correlations between BOLD response to the N-Back working memory task and Ki^{cer} in either group or between the groups.   This did not support hypothesis 3.

Hypothesis 4:

Antipsychotic treatment would increase the neural activation in DLPFC and that the change will be directly correlated to improvements in negative symptoms.

I tested this hypothesis in the study presented in chapter 4. The results were that with treatment, there was no significant change in activation from baseline to follow-up nor were there any significant correlations between a change in negative symptoms and a change in BOLD response during a higher working memory load. This did not support hypothesis 4.

5.1. The neural basis of working memory in schizophrenia and the link to negative symptoms

Most previous studies investigating working memory in schizophrenia have included patients who were already on antipsychotic medication and have not controlled for performance on the working memory task [294, 437, 457, 461, 476, 479, 481, 482]. Hyperactivity in the thalamus was report in a previous study of medicated first episode patients with a very small sample size of n=9 [437]. However, antipsychotics can affect BOLD signal [277, 280, 500] by directly modulating neuron activity and altering cerebral blood flow [274, 275] to affect fMRI responses and signal, meaning that it is difficult to draw conclusions from studies in medicated patients [272, 273], although in experimental chapter 4 in this thesis, treatment did not produce a significant difference between baseline and follow-up time points. A strength of the results from chapter 2 thus is that they are in unmedicated first episode patients. My results add to previous findings by confirming hypofunction in hippocampus and thalamus in treatment-free first-episode patients [457, 476] and [294, 296] respectively. There was no group difference in DLPFC activation, which extends the findings of one previous paper [457] by showing that the neural response in the DLPFC is not altered during working memory performance in unmedicated patients. However, they contrast with findings from other papers that have shown hypofunction in the DLPFC in drug free patients. Importantly, the sample sizes in these previous studies were smaller by more than 50% [296] or 60% [294] smaller than my sample or were in medicated patients [296]. False

positive rates are high in studies with small sample sizes (see review by Mufano et al. (2017) [564]). Thus, the positive findings in these previous studies may represent a false positive.

Another factor to consider is performance on the task, whereby group differences in BOLD may reflect performance differences rather than altered neural responses during the task. My findings disagree with much of the literature on neural activity during working memory tasks in schizophrenia, which have generally reported prefrontal hypofunction [294, 296, 479, 481]. However, these previous studies did not control for performance on the working memory task [294, 457, 479, 481]. By not controlling for task performance, conclusions previously drawn might be due to performance differences, rather true neural activity response differences between patients and healthy controls.

A further difference between my study and most previous studies is that they compared the working memory condition with an attentional control condition [294, 296, 457, 461, 476, 479, 481, 482, 501], whereas my study examined the effect of working memory load. Contrasting 2-Back with 1-Back, conditions that show greater similarities, are comparable with respect to the task requirements and both activate working memory, whereas the contrast with an attentional control is not comparable. Thus, hypofunction in the previous studies could reflect increased neural activity during the attentional control rather than reduced response during working memory.

It is important to recognise the possibility of a type II error, that is failing to reject the null hypothesis, for the null findings in Chapter 2. In Table 30 I summarise the sample sizes of the fMRI studies that have investigated working memory in schizophrenia.

Table 30: Sample Sizes

Study	Sample Size FEP/HC	Medicated?
Current Study	29/30	No
Nejad et al. (2011) [474]	23 / 35	No
Mendrek et al (2004) [294]	10/8	Yes
Meisenzahl et al. (2006) [296]	16/12	No
Schneider et al. (2007) [457]	75/81	Yes
Guerrero-Pedraza et al. (2012) [476]	30/28	Yes
Smieskova et al (2012) [479]	21/20	Yes
Vogel et al. (2016) [461]	22 / 20 (Chronic Scz)	Yes
Crossley et al. (2009) [481]	10/13	Yes
Nielsen et al. (2017) [482]	17/18	Yes

The sample size of the paper in chapter 2 is the second largest of the studies listed in Table 30, and the largest of unmedicated patients. The largest was part of a multi-site study and was therefore able to recruit 156 participants (78 patients) [457]. I used G*Power to calculate the sample size a future study would need to be to detect a significant group difference. I based this on the effect in my primary regions of interest using the means of the beta group differences for an independent t-test with alpha<0.05 and power>80%. The betas were extracted via MARSBAR, and I calculated the means and effect sizes for each group in SPSS, version 24.

The effect size from the DLPFC was d= 0.39 and would therefore require n = 210 (105 per group) to achieve the power required to detect likely effects. A multi-site study would probably be required to recruit a sample size of this magnitude. The hippocampus had an effect size of d = 0.97, requiring n = 56 (28 per group), and the thalamus had an effect size of d = 0.96, requiring n = 60 (30 per group). Overall, whilst I cannot exclude a small effect in the DLPFC, the major alterations in neural response during a working memory task in psychosis were in the thalamus and hippocampus. I discuss the implications of this in the section below.

Chapter 2 provides new evidence to show that lower activation in patients was not directly associated with greater severity of negative symptoms. Previous papers to investigate this included medicated first episode patients, which the paper in Chapter 2 did not [461]. This extends the findings of one previous paper by demonstrating that working memory BOLD response is not related to negative symptoms in medication-free patients [474]. This paper, however, looked at the difference between default mode network activations and deactivations during a verbal N-Back task compared with chapter 2 which looked at neural engagement during increased working memory load (i.e.: the 2-back relative to the 1-back condition).   Although my findings are in agreement with this relationship, Nejad et al [474] did not look at the BOLD response in the same way, and so the findings are not directly comparable.

In conclusion, BOLD response during performance on a working memory task is lower in first episode psychosis, free from the effect of antipsychotic medication, in thalamus and hippocampus but not significantly altered in the dorsolateral prefrontal cortex. This indicates altered neural function in the thalamus and hippocampus in working memory impairments in psychosis. Moreover, cortical response is not related to negative symptoms, which indicates that

this part of cortical function does not underlie negative symptoms. It further suggests that the neural source of working memory impairments in schizophrenia is different to that of the underlying negative symptoms.

5.2. The link between striatal dopamine and the neural basis of working memory in healthy volunteers and schizophrenia

The paper presented in chapter 3 extends previous studies by being the first to test the relationship between striatal dopaminergic measures, and N-Back BOLD response, in first-episode patients. Previous papers looked at the relationship between cortical neural response during cognitive tasks and dopamine synthesis in chronic [326] and in at-risk [139] patients, but not first episode. A limitation of the study of at-risk subjects is that not all at-risk patients will go on to develop schizophrenia [325]. The implications from this are that the findings might not be relevant as they were not specific to schizophrenia, only to those who are at-risk of developing schizophrenia. The paper examining the relationship between DSC and BOLD in chronic patients used the Wisconsin Card Sorting Task (WCST). Two potential confounding factors in this study include long-term medication as antipsychotics act on the dopamine system [565] and illness chronicity. Notwithstanding these factors, the difference in findings between my study and this study may be due to differences in the tasks used. The WCST is a test of executive function [566], whilst the measure that I used was a working memory task. Thus, taken with the prior results, the lack of relationship I found may indicate that whilst neural response during working memory is not related to striatal dopaminergic function, cortical activity during executive function is linked to striatal dopaminergic function.

A limitation of Chapter 3 was that dopamine was only measured in the striatum. The FDOPA tracer does not reliably measure prefrontal cortex dopamine synthesis capacity and reports in the

PFC are uninterpretable [567]. It is possible that although there was no relationship with striatal dopamine and BOLD, there might have been with PFC dopamine and BOLD. To investigate this, a study using a tracer that measures dopamine in the PFC, such as, D1R agonist [$_{11}$C]SCH23390 [568], and correlating that with the BOLD response during a working memory task is required. Alternatively, using a combined PET/MR scanner would be ideal to capture the response to the working memory task and directly correlated with dopamine synthesis capacity in the PFC.

It is important to consider potential sources of error in the measurement that may contribute to the null finding in chapter 3. There are some factors that can increase the noise in the PET measurement, which might explain the null finding such as movement, anatomical differences, and partial volume. Head movement can have a pronounced effect on the amount of activity measured in the ventral striatum [569]. It can be corrected for, but realignment is restricted and not able to correct for movements that happen within frames [570]. Individual differences in anatomy may lead to registration errors during the quantitative measure of the ventral striatum [570]. Thirdly, the resolution constraints of PET images may mean that small region of interest volumes, for example from the ventral striatum, is especially vulnerable to underestimation of radioactivity concentrations due to spill-over effects and partial volume [569].

I will now detail what I have done to reduce the impact of these factors. To minimise head movement, a head rest and straps were used, whilst a frame-by-frame movement correction was employed. Anatomical differences were reduced by using SPM8 to normalise the ROI map together with a ([^{18}F]-DOPA) template [565, 570] to each individual PET summation image. This nonlinear transformation process permitted ROIs to be automatically put on individual [^{18}F]-DOPA PET dynamic images. Finally, to reduce the effect of anatomical individual volumetric differences, partial volume correction can be used by co-registering the PET images onto high resolution

magnetic resonance images using algorithms. I do not think that these disadvantages explain my

results, due to the steps that I have taken to minimise these shortcomings including head rest and

straps, frame-by-frame movement correction and partial volume correction. In conclusion my

findings indicate that cortical activation in working memory is not related to striatal dopamine in

first episode psychosis patients.

5.3. The effect of antipsychotics on neural function during working memory performance

The paper in chapter 4 extends previous findings by controlling for chronicity and working

memory task with the inclusion of treatment-free first episode patients prior to commencing

treatment and repeat scanning post-treatment. There are very few longitudinal studies looking at

the effect of antipsychotic treatment on brain function and whether this is related to clinical

change. Chapter 4 provided new evidence that there were no BOLD differences during a working

memory task in activation from baseline to follow-up time points; therefore, antipsychotic

medication did not significantly affect the neural response during the N-Back working memory

task. It also extended previous findings [292] by demonstrating that a change in BOLD response

from the working memory task was not linked to clinical change but in disagreement with

another first episode study [292] and one in chronic patients [298].

In the follow-up study in Chapter 4, one limitation was the inclusion of minimally treated patients

at baseline. I wanted to be consistent with the baseline papers by including only treatment-naïve

and treatment-free patients. Unfortunately, the sample size (n=16) was relatively small in

Chapter 4 and with the removal of the 4 minimally treated patients, the power would have been

decreased with an overall n = 12. Those patients with the most severe negative symptoms often

do not volunteer to take part in research. It should be recognised that due to the naturalistic

nature of the study, the 16 patients at follow-up were on 7 different types of antipsychotic

medication: Amisulpride, Aripiprazole, Lurasidone, Olanzapine, Paliperidone, Quetiapine and Risperidone. This may have added variability if they have different effects on neural response, reducing my power to detect a significant effect. Whilst it would be useful to just include patients on one drug, this would have reduced the generalisability of the results.

Another limitation was the possibility of practice effects, as it has been demonstrated that continuous practice may have an effect on activation of the PFC during the N-Back task [561]. However, Van Veelen et al (2011) confirmed that practice effects did not impact on their results [292]. This is relevant to my study as the results that I presented in chapter 4 were unlikely to be due to practice effects, but rather that antipsychotics do not have an effect the neural response to a working memory task. In a future study I would include a healthy control group to have two scans at similar time points to patients to fully investigate whether there was a practice effect. Nevertheless, practice effects are unlikely to explain my null results as they would be expected to alter the neural response.

Chapter 4 was a naturalistic study whereby the clinical team, in discussion with the patient, made the choice of antipsychotic medication. I was not able to test the effect of one specific antipsychotic and therefore must accept that some antipsychotic medication used in this paper (e.g., amisulpride) have more selectivity for D_2 receptors than other antipsychotics. Therefore, the absence of an effect of treatment on neural activation should be interpreted with caution possibly due to the different antipsychotics that the patients were on. It is intrinsically difficult to separate real antipsychotic treatment effects on negative symptoms from spontaneous fluctuations in negative symptoms related to the natural course of the illness. Including an untreated patient control group would be required to disentangle these factors but has ethical implications as it requires not treating patients.

In conclusion, these findings indicate that antipsychotic medication does not have a significant impact on neural pathways underlying working memory impairment in psychosis.

5.4. General Limitations of the approaches taken in my thesis

5.4.1. Clinical

All three studies included first-episode of psychosis patients, which may have included patients with affective psychoses as well as schizophrenia. It is recognised that diagnosis can change over the first few years of illness with only 33% of patients having the same baseline and lifetime diagnosis of schizophrenia (ICD10) [556]. Consequently, my results may not be specific to schizophrenia and further follow-up is needed to determine the final diagnoses in the sample and if my results are specific to schizophrenia.

The PANSS scale was used to assess negative symptoms in keeping with previous studies [295, 474]. The problem with the PANSS for assessing negative symptoms, is that it includes items on the negative sub-scale that are not negative symptoms such as *Difficulty in Abstract Thinking* and *Stereotyped Thinking,* which might dilute the measure of the severity of negative symptoms. To overcome this limitation, the negative dimension of Marder's Five Dimensions of Schizophrenia scale [510] was also used in chapter 2. The Marder scale removes abstract thinking and stereotyped thinking from the PANSS negative symptom factor score to provide potentially a more valid measure of negative symptoms [510]. There are more sensitive "next-generation" [518] scales available to measure negative symptoms such as the Brief Negative Symptom Scale (BNSS [571, 572]) and the Clinical Assessment Interview for Negative Symptoms (CAINS [573, 574]) could potentially more accurately measure negative symptoms. Both the CAINS and BNSS have good discriminant validity, convergent validity and internal consistency [518], but the CAINS takes approximately 30 minutes to administer whilst the BNSS takes 10-15 minutes [575].

In this thesis, patients' negative symptoms were relatively moderate, and this may have reduced power to detect relationships. The patients were not recruited for their negative symptoms, the focus of recruitment was to enrol patients into the study who were treatment-naïve or treatment-free and would shortly be started onto medication. The lack of relationship between negative symptoms and BOLD could be because this cohort only included first episode patients and therefore did not have very pronounced negative symptoms yet. To remedy this, the focus of future studies, could focus on recruiting unmedicated patients with more severe negative symptoms. The moderate negative symptoms of the patients in my sample were comparable to other first episode studies [295, 461], indicating it is likely to be representative of patients in general, and particularly first episode patients. Additionally, as the illness progresses, negative symptoms become more pronounced [576].

5.4.2.   Limitations of fMRI

The first limitation of fMRI is that it gives an indirect measure of brain activity through changes in blood vasculature that accompany the neuronal activity. Neuronal activity is systematically linked with changes in the comparative concentration of oxygen in local blood supply [577, 578]. Oxygenated blood has different magnetic susceptibility compared to deoxygenated blood and it is these changes in the ratio of oxygenated/de-oxygenated blood (i.e. haemodynamic response function) that can be inferred from fMRI by measuring the BOLD response [579]. Secondly, fMRI has limited temporal resolution in relation to neural activity because the haemodynamic response is extended over time by 4-6 seconds and temporal smoothing makes it difficult to locate the precise moment of activity [311]. Thirdly, another weakness is the inability to distinguish neuromodulation, excitatory and inhibitory inputs from the total neuronal mass action [311].

Other limitations of fMRI relate to the participants and hardware. The first of these is artifacts caused by physiological issues (heart rate and respiration); fMRI noise from the gradient and magnetic field; head movement; thermal motions of free electrons. These can also appear in the data as high-frequency spikes or low-frequency drift over time. These issues can be minimised by choosing the correct parameters for the paradigm, specialised sequences such as spin-echo and minimising head movement [580]. Gradient echo pulse sequences are suited to faster imaging as the echo time is shorter therefore allowing for faster signal acquisition [581] and that is why it was chosen to be used in this thesis over spin-echo sequences.

Head movement was minimised in this thesis by including scan-nulling regressors to model periods of large volume-to-volume motion (movement spikes); and, if there were more than 5 spikes (over 0.5mm) during the task, participants were removed from further analysis. During analysis, I thoroughly checked the data for motion artifacts and movement spikes, and outliers were removed. Movement can be corrected by removing the movement "spike" and the two slices either side of the spike. In this thesis, the entire participants' data was removed if they moved too much; defined as a frame-wise displacement threshold of 0.5mm or greater as anything under this can largely be corrected by existing regression strategies [507], to minimise the effect of this. Despite removing participants' scans if there were more than 5 spikes (over 0.5mm) during the task, patients still made more small movements during a scan than healthy controls and there were differences in head movement between the groups. Thus, whilst large movement effects are unlikely to influence my findings, I cannot exclude an influence of group differences in movements less than 0.5mm on my data.

Cluster-extent based thresholding is a common method for multiple comparisons corrections of statistical maps in neuroimaging studies. This is due to its high sensitivity to signals [582]. It does

provide however low spatial specificity, whereby researchers can only infer that there is a signal *somewhere* within a significant cluster and therefore cannot make inferences about the statistical significance of specific locations within the cluster. Woo et al. (2014) states that this is a problem when researchers use a liberal cluster-defining primary threshold with a liberal p-value (i.e., $p<0.01$) as it can produce large clusters covering multiple anatomical regions and it can increase the false positive rate [583]. This means that it is impossible to reliably deduce which anatomical regions show actual effects [583].

Cluster-extent based thresholding detects statistically significant clusters based on the number of contiguous voxels where the voxel-wise statistics values are above a predetermined primary threshold. Statistical tests control the estimated false positive probability of the whole region instead of controlling the estimated false positive probability of each voxel. Cluster-extent based thresholding usually consists of two stages [582, 584]. Firstly, an arbitrary voxel-level primary threshold defines clusters by keeping groups of suprathreshold voxels. Secondly, a cluster-level extent threshold (k, the unit of contiguous voxels), is determined based on the estimated location of clusters under the null hypothesis of no activation in any voxel in that cluster.

There are advantages of cluster-extent based thresholding. Firstly, voxel-level corrections for multiple comparisons are still FWE correction implemented under gaussian random field theory with all the assumptions required therein, typically that the map can be treated as a uniformly smooth field. It is very stringent so that they could markedly increase Type II errors (i.e., low sensitivity) unless sample sizes are very large [585]. In contrast, cluster-extent based thresholding has quite high sensitivity [582, 586]. Secondly, cluster-extent based thresholding considers the fact that individual voxel activations are not separate from the activations of their neighbouring voxels particularly when the data have been spatially smoothed [587, 588].

Regardless of these advantages, cluster-extent based thresholding also has some limitations, particularly low spatial specificity when clusters are larger [582, 589]. The cluster-level p-value does not affect the statistical significance of activation at a particular location or voxel(s) within the cluster. Instead, it explains the probability of getting a cluster of a given size or larger under the null hypothesis. The larger the clusters become consequently the less spatially specific the inference. If cluster sizes are tiny enough and are within a single anatomical area of interest, then cluster-extent based inferences are fairly certain [583]. If, however, a liberal primary voxel-level threshold (e.g., p<0.01) is chosen to define clusters, clusters that survive a cluster-extent based threshold for a family error wise rate correction regularly become large enough to cross anatomical borders. This liberal primary threshold has the disadvantage in the spatial specificity of anatomical or regional claims that can be made [583]. If significant clusters cross many anatomical boarders, the results produce very little useful neuroscientific information nor if a single cluster covers two anatomical regions, the researchers cannot make inferences about a specific region [583]. Instead, they should only infer that the signal is somewhere within the cluster [583]. Cluster size is critical to making the cluster-extent thresholded results interpretable and valuable in building a collective understanding of human brain function [583].

The cluster-forming statistical height threshold for the analysis in all three chapters is p<0.001 uncorrected, which is in line with the recommendation from Woo et al. (2014) who recommend setting p<0.001 as a lower limit default [583]. The family-wise error corrected p-value was used for both the threshold level for whole brain and peak level for ROI analysis. I therefore believe that the analysis satisfied these criteria.

Some papers report that neural activation during the processing of working memory is illness duration and gender-specific in patients with schizophrenia [590]. In my PRISMA review on

average, 75.38% (s.d 15.17) of participants were male, with one study exclusively using only male

participants and one study not reporting gender. Elsabagh et al (2009) [590] reported that

duration of illness was related to reduced DLPFC activity in male participants and decreased

cerebellum activity in female participants. Sex-specific effects of illness duration were also seen

in the inferior frontal and superior temporal gyri (in females) and the inferior parietal cortex (in

males). Elsabagh et al (2009) [590] detected no significant effect of ageing on working memory

neural activation in patients, thus concluding that a longer duration of illness in people with

schizophrenia has sex-specific associations within the working memory neural network, with

expected association between illness duration and impaired PFC activation apparent in male, but

not in female patients. I have matched for age and gender in my studies, but my studies were not

designed or powered to test for a group-by-age or group-by-gender interaction and so I did not

conduct this. A future study with a sufficiently large sample size to permit testing for interactions

would be useful to address this.

5.4.3.   Working Memory Task

One could also argue that these studies might have been limited by the choice of working

memory task, as it might be "nonmonotonic" [553]. The normal response from the DLPFC to

parametric variations in working memory load is an "inverted-U" [436, 554] (see Figure 72). The

first graph shows patients and healthy control participants operating on the same working

memory curve until the patients become "hypofrontal" when they reach their working memory

capacity limit sooner than controls. The second graph shows that patients and controls are on

different curves with patients peaking on the U sooner than controls, therefore appearing

"hyperfrontal" at lower working memory loads. When their memory capacity is reached,

"hypofrontality" results [436]. This model predicts that patients and controls might reach the

same magnitudes of prefrontal activation but at different working memory load levels. Many

have argued that task-related activation patterns are a non-linear and the inverted U-shaped function that relates the fMRI signal to working memory load is shifted to the left in patients with schizophrenia [591]. It has been suggested that the discrepancies in the literature could be related to task requirements or poor task performance in schizophrenia [436, 555]. Alternatively, it might be related to the u-shaped curve where the patient curve is shifted to reflect lower capacity [442].
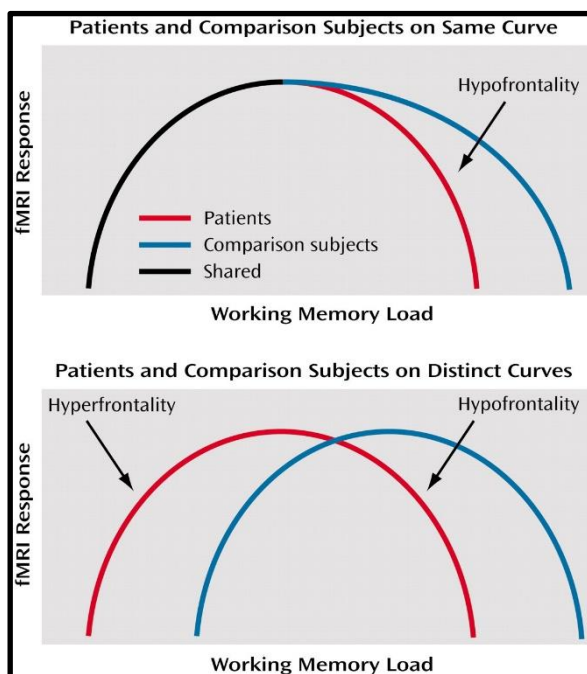
Figure 72: Inverted-U-Shaped Curve Representing Theoretical Response of the Dorsolateral Prefrontal Cortex to Increasing Working Memory Load in Patients with Schizophrenia and Healthy Comparison Subjects (From Callicott (2003) [436])

The limited number of steps or loads with N-Back that I used might have restricted the ability of the task to show an inverted-U. Future work could select a different task that taps working memory such as the ability to generate and monitor a sequence of responses (e.g., self-ordered

working memory task (SOWT as used by [553]). The pictures presented in the SOWT task, are presented in different spatial arrangements on each trial and participants are required to point to a different picture every time. The test requires the ability to organise and carry out a sequence of responses as well as to retain and constantly monitor the responses made.

In contrast to other N-Back working memory papers, reaction times were not presented; and accuracy was only controlled for as a measure of performance in this task. Onset times were modelled in the first level for each participant. Reaction time did not reflect performance, as, in all levels except the 0-back level, subjects knew that the next response would be presented before they were allowed to respond, as was determined by the preceding stimuli [592]. For this reason, participants were instructed to concentrate on accuracy rather than speed, as has been described elsewhere [593].

The accuracy rate that was set in these experimental chapters was 50% N-Back task, which is achievable and the rate is higher than chance performance (25%) [592]. However, in addition to this, onset times, correct responses, false alarms, and misses were all modelled in the first level analysis. Due to the block design of this study, I was not able to investigate different working memory components and sub-processes, although patients with psychosis show different deficits during encoding and retrieval of information [186, 497, 517]. This could be done by using the Memory Updating Task which looks at retrieval, transformation, and substitution, and can isolate these components from other processes [594].

As discussed in the introduction, there are quantitative and qualitative differences in the cognitive processes between 2-back and 1-back. The 2-back condition requires longer storage

duration of the stimuli and more processing time than 1-back because participants must compare

each stimulus to the item that was seen or heard two items ago for future comparisons.

Nevertheless, although the storage and rehearsal demand of the 1-back task is reduced relative

to the 2-back condition, the 1-back still requires most of the same cognitive processes

constituting working memory seen with the 2-back. The one exception is the 2-back condition

requires temporal ordering of stimuli, which is not required in the 1-back condition. Thus, the

contrast between the 1-back and 2-back conditions includes both quantitative increases in a

number of cognitive processes, including working memory, and an a qualitative difference in

cognitive processes, as temporal ordering is required for the 2-back and not 1-back conditions.

There are some discrepancies in the literature regarding the relationship between N-Back load

and brain activation. As examined in the introduction, studies mostly report linear patterns in the

PFC and DLPFC as the memory load increases from 0 to 3 back (e.g., [467, 471]). However, it

should be recognised that there are exceptions. For example, a study using functional MRI

reported almost no difference in DLPFC activation from 0 to 1 back or 2 to 3 back but a large

increase in activation from 1 to 2 back [472]. It could be suggested from this paper, that the

neural processes involved in the 1-back load are more similar to those that are used in the 0-back

condition, sometimes referred to as choice task [473], than to the 2- and 3-back conditions.

5.5. Implications for understanding the neurobiology of working memory impairments in schizophrenia

5.5.1.   BOLD function during working memory

The implication from the results presented in this thesis is that the DLPFC function is not impaired

during working memory in first-episode patients with schizophrenia, but hippocampal and

thalamic function is as previously reported [188, 222, 326, 408-440]. In contrast, I found that

patients had displayed blunted load-related (2-Back>1-Back) responses in the thalamus and hippocampus. The lower neural activation in these regions but unchanged in the DLPFC, suggests that the hippocampus and thalamic part of the circuit is altered but not the DLPFC. Andreasen et al. (1999) reported that the cortical–thalamic–cerebellar cortical circuit is believed to be abnormal in patients with schizophrenia [354]. The results from Andreasen et al (1999) and the current chapters might reflect inefficient processes or compensatory strategies in schizophrenia. These might be the result of an imbalanced system trying to manage differentially with the requirements of the task.

In working memory, the thalamus is vital for maintaining memories [349] working memory [350, 351] and verbal fluency [347]. The involvement of the hippocampus in working memory is important in the storage of memories and formation of associations [379], encoding, retrieval, novelty detection and successful retrieval [380, 381]. Both regions have abnormal function in schizophrenia. The literature reports both hypoactivity [294, 413, 496-498, 595, 596] and hyperactivity [437] in the thalamus but these inconsistencies maybe due to cognitive performance or cognitive load. The thalamic abnormalities in schizophrenia might be the product of a compensatory mechanism for working memory deficits [597]. The same inconsistencies with hyperactivity [230, 239, 481] and hypoactivity [424, 598] have also been shown the hippocampus in schizophrenia.

Poor performance on working memory might be related to related to hypoactivation in the hippocampus [458] or retrieval, storage and maintenance impairments [457]. Hypoactivation is the reduction in or less than normal activation. One potential explanation for this is there is less recruitment of neurons during the task in patients, for example due to lower levels of cortical dopamine signalling (as discussed in the introduction). An alternative explanation is that it reflects

altered inputs into the region. Striatal dopamine activity can modulate working memory performance [228] and there is evidence in schizophrenia that prefrontal activation alterations are related to striatal dopamine dysfunction [229]. Experimental evidence that striatal dopamine alterations could affect working memory comes from a study of mice with a selective overexpression of D_2 receptors in the striatum. These mice show decreased performance on working memory tasks, indicating that increased striatal dopamine neurotransmission can impair working memory putatively by altering cortical inputs [154]. In addition, a study of people with schizophrenia investigated the relationship between striatal dopamine synthesis capacity and activation in the prefrontal cortex during a cognitive task [326]. This study found higher striatal dopamine synthesis capacity was associated with lower activation in the prefrontal cortex during the cognitive task in patients with schizophrenia. To my knowledge, this is the only study that has investigated the relationship between striatal dopamine synthesis capacity and prefrontal activation during a cognitive task in people with schizophrenia prior to my study.

Another theoretical consideration of hypoactivation in the working memory system is that hypoactivation can either be task-related underactivity or be due to greater baseline activity. In the current study, there were no significant behavioural differences between patients and controls on task performance but there was hypoactivation in the thalamus and hippocampus in patients relative to controls at baseline, that is greater difference baseline activity in controls. Both the thalamus and hippocampus have abnormal function in schizophrenia, although the literature is inconsistent, with studies reporting hypoactivity [294, 413, 496-498, 595, 596] or hyperactivity [437] in patients with schizophrenia relative to healthy controls. This inconsistency might be due to how well the participants understand the task, cognitive performance, task difficulty or cognitive load. The thalamic abnormalities in schizophrenia might be the product of a compensatory mechanism for working memory deficits [597]. Poor performance on working

memory might be related to related to hypoactivation in the hippocampus [458] potentially

leading to retrieval, storage and/or maintenance impairments [457].   In conclusion, the

hypoactivation I report could result from decreased function of the specific regions, for example

due to reduced recruitment of neurons during the task, or an impairment in inputs from other

regions. It is not possible to disentangle these possibilities using the methods I employed in this

thesis.

Another factor to consider in relation to the abnormal function in the hippocampus and thalamus

is glutamate. The glutamate system sends out projections from both the thalamus to the cortex

and hippocampus to the striatum in the human brain [168]. Glutamatergic neurotransmission in

the hippocampus might be related to abnormal dopaminergic neurotransmission in schizophrenia

and psychosis [72]. Finally, it should be noted that identification of a significant regional response

does not suggest that this region is exclusively or more strongly involved in the process of interest

compared to other regions, more that, whilst the null hypothesis has been rejected in this region,

it has not been so refuted elsewhere.

5.5.2. Neural Response during Working Memory Impairment and Negative Symptoms

The findings presented in this thesis suggest that abnormal hippocampal and thalamic functioning

could underlie the working memory impairments seen in unmedicated FEP but are unlikely to be

related to the generation of negative symptoms. The key implication is that the neural response

during working memory and negative symptoms may not be related and might be different from

those originally proposed in previous research. Therefore, the mechanism underlying these

different symptoms is different and by extension one treatment will not address both. Because

my sample was first-episode patients, there is a reduction of the impact of factors such as long-

term medication, disease chronicity, or social withdrawal on negative symptoms. This means that my studies show a true representation of effect of lower hippocampal and thalamic activation on the BOLD response during a working memory task at this early stage in the course of the illness along with the fact that might be different mechanisms for the working memory and negative symptoms, which is also seen during the first episode of schizophrenia.

Studies that have investigated the neurobiological basis of negative symptoms have looked at two main areas: the ventral striatum and DLPFC. In some papers, the relationship between ventral striatum activation and negative symptoms seems shows an association [599-601] whilst other publications did not [602-604] finding instead a relationship between positive symptoms and the ventral striatum. In a recent meta-analysis of 25 task-related functional magnetic resonance imaging studies in schizophrenia (see [605]), negative symptoms were not related to dorsolateral prefrontal activity but the ventrolateral PFC and ventral striatum were moderately correlated with negative symptoms. The authors suggest that these conflicting findings can be explained by considering several factors, such as medication status (treatments with first and/or second-generation antipsychotics or no medication), patients' diagnosis (schizophrenia or schizoaffective disorder) and assessment instruments used to rate psychopathology. Additionally, the inconsistencies might also be due in part to the heterogeneity of negative symptoms [606] or that the tasks may not capture key aspects of negative symptoms such as lack of volition. Future research could investigate the underlying neurobiological mechanisms of both negative symptoms as a cluster and of the individual negative symptoms.

5.5.3. Dopamine and PFC

My findings showed there were no significant correlations between BOLD response to the N-Back working memory task and Ki^{cer} in either group or between the groups. This implies that striatal dopamine function is not significantly related to cortical function during working memory performance. This suggests that the mechanisms underlying altered thalamic and hippocampal responses during working memory performance are different to those underlying striatal dopamine function. My findings contrast with previous findings of a relationship between higher striatal dopamine synthesis capacity and lower prefrontal activation during a cognitive task [326]. The previous study included only six patients, whereas mine included 24 patients. Given that false positive results are more likely with small sample sizes, it is possible that the prior result was a false positive [607]. However, one difference between my study and the previous one is that the patients in my study were in their first episode, whilst those in the previous study were chronically unwell. Thus, it is possible that effects are more marked in chronic patients than in first episode patients. A longitudinal study is needed to test this possibility.

It is important to recognise that cognitive impairment might still be linked with dopamine in the prefrontal cortex (PFC) as I did not measure dopamine in the PFC, particularly as blockade of the mesocortical dopamine pathway has been associated with cognitive symptoms [227]. Further evidence for a role of cortical dopamine comes from brain imaging studies in patients with schizophrenia which have shown deficits in cortical activation during cognitive tasks following dopamine antagonist administration [187, 608]. A post-mortem study showed lower tyrosine hydroxylase immunolabeling, which is a marker of dopamine innervation, in the DLPFC in schizophrenia, suggesting there is lower cortical dopamine function in schizophrenia [609]. Dopamine is known to have a role in elevating the signal-to-noise ratio in the cortex by interacting with both D_1 and D_2 receptors [399]. In preclinical data, suboptimal or excessive

stimulation of D_1 receptors may produce working memory deficits [177, 183, 610]. D_1 receptor antagonists impair DLPFC working memory performance in monkeys [183], whilst full D_1 agonists SKF81297 and A77636 can partially reverse the deficits in spatial working memory [177]. This evidence is suggestive of the importance of developing D_1 agonists as an adjunctive treatment for cognitive impairment in schizophrenia.

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If striatal dopamine does not underlie my findings, then it might be prudent to consider the role of hippocampal and thalamic glutamate. In tracing studies, cortical projections to the striatum show a topographical distribution across the striatum [103, 166, 167] with projections from the hippocampus, projecting to the anterior and ventral regions of the striatum. Glutamatergic neurotransmission in the hippocampus might be related to abnormal dopaminergic neurotransmission in schizophrenia and psychosis [72] as there are extensive projections from

glutamatergic output neurons in the hippocampus to the striatum in the human brain [168].

Information processing is enabled by cortico-striatal-pallidal-thalamo-cortical loops. The striatum

sends inhibitory GABAergic projections to the pallidum that it itself projections to the thalamus.

The thalamus sends glutamatergic projections back to the cortex. Stronger activations in the

thalamus in patients with schizophrenia might be the product of a compensatory mechanism for

working memory deficits [597].

5.5.4. Antipsychotic Treatment

Antipsychotic treatment did not influence neural response during the N-Back working memory

task, nor was a change in BOLD related to any clinical change. The implication of this finding is

that marked working memory impairments, seen from the onset of psychotic disorders, continue

to have a limited response to current treatments [524]. My understanding has been advanced by

the fact that the therapeutic effects of antipsychotic medication do not alter or "normalise"

working memory and subsequent neural response. The results from this dissertation identify the

hippocampal and thalamic function as potential targets for the development of treatments to

improve working memory performance in psychosis. Understanding these mechanisms is

important to help identify new treatment approaches [174]. Further work is required to

determine the nature of neurobiological alterations that could be targeted, although other work

indicates that glutamatergic and GABAergic alterations could be involved [525, 527, 530].

Additionally, future studies could look at individual classes of antipsychotics (first or second

generation, for example) and investigate where these classifications have an impact on BOLD

signal as experimental chapter 4 did not differentiate between medication classifications.

5.6. Future Directions

As discussed in the limitations section above, my study measured dopamine in the striatum, but it did not measure it in the cortex. Thus, it remains unknown if dopamine in the prefrontal cortex is altered in first episode psychosis and if it is altered is it related to working memory impairment. A possible direction future research can take, is using combined PET/MR machines to look at dopamine in the PFC during a working memory task. Combined scanners have the potential to show specifically that alterations in the BOLD signal response to working memory task are in brain regions that are the result of simultaneous and co-located with decreases in dopamine release. Initial PET–MRI studies have already demonstrated that these complementary measures of brain function can provide new insights into the structural and functional organization of the brain [611].

One way to measure cortical dopamine might be to employ the same tracer that Slifstein et al. (2015) used ([^{11}C] FLB^{457}) to measure amphetamine-induced dopamine release in the DLPFC in patients with schizophrenia [192]. They found that patients with schizophrenia demonstrated, in vivo, a blunted amphetamine-induced dopamine release in the DLPFC [192]. This implies that the capacity for dopamine release in the DLPFC in schizophrenia might be a more widespread deficit extending to many cortical and extrastriatal regions, including the midbrain. It further suggests a differential regulation of striatal dopamine release in the associative striatum compared to regions outside of the striatum. Slifstein et al. also found an association between activation of the DLPFC from the self-ordered working memory task (as measured by BOLD) and the index of DA release capacity, suggesting that blunted release might impact frontal cortical function. Slifstein et al [192] has been the only *in vivo* study measuring dopamine capacity in the prefrontal cortex in schizophrenia [192]. The main reason for this is the lack of a suitable tracer for cortical dopamine release. Radiotracers, for instance carbon 11–labelled- (+)-PHNO and carbon 11–

labelled raclopride, are suitable for detecting acute fluctuations in striatal dopamine levels [192], but due to the limited anatomical distribution and low density of dopamine receptors in the cortex [612], these reasons prevent their use for imaging of dopamine receptors in the cortex.

The significant finding in this thesis of lower activity in thalamus and hippocampus, could be expanded to look at working memory impairment in these regions and its relationship with the glutamate system. Blocking glutamate-medicated excitatory neurotransmission by NMDA receptor antagonist can mimic cognitive and negative symptoms (as well as positive) in schizophrenia. This suggests that increasing NMDA receptor transmission may reverse cognitive deficits [613, 614]. NMDA receptor ablations on GABA interneurons impairs hippocampal theta rhythm causes working memory impairment [615]. With magnetic resonance spectroscopy scans, thalamic glutamine has been elevated in patients with schizophrenia [616]. These findings suggest that glutamate function is integral to working memory performance. By exploring the pharmacology of working memory impairment, future research could concentrate on developing much needed treatments for cognitive and negative symptoms of schizophrenia.

5.7. Conclusions

In chapter 2, I showed that those patients had hypofunction in the hippocampus and thalamus compared to healthy controls during a working memory task, but this was not related to negative symptoms. In Chapter 3, I demonstrated that altered brain responses in these regions were not linked to striatal dopamine synthesis capacity. Chapter 4 showed that antipsychotics do not improve neural response measured with BOLD during a working memory in patients experiencing their first episode of psychosis.

These findings indicate that thalamic and hippocampal dysfunction could underlie working memory impairments in first episode psychosis, but that this is not linked to negative symptoms or striatal dopamine function. Moreover, they provide preliminary evidence to indicate that antipsychotics do not alter neural responses during working memory performance. These findings highlight the potential role of thalamic and hippocampal dysfunction in cognitive impairments in psychotic disorders, and suggest further work is required to understand the underlying pathophysiology. Ultimately this could help the development of treatments for cognitive impairments in psychosis.

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