Functional and Structural Brain Imaging

Correlates of Cannabis Use in Young People

with Schizophrenia

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STATEMENT OF ORIGINALITY

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Martin Cohen

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ABSTRACT

Converging evidence from epidemiological, clinical and neuropsychological research suggests a link between cannabis use and increased risk of psychosis. Long-term cannabis use has also been related to deficit-like "negative" symptoms and cognitive impairment that resemble some of the clinical and cognitive features of schizophrenia. The current brain imaging study investigated the impact of a history of heavy cannabis use on impaired executive function and cerebellar brain structure in first-episode schizophrenia patients. Functional brain imaging data were collected whilst study participants were performing the Tower of London task in a magnetic resonance imaging scanner. Event-related blood oxygenation level-dependent (BOLD) brain activation was compared between four age and gender-matched groups: 12 first-episode schizophrenia patients; 17 long-term cannabis users; 7 cannabis using first-episode schizophrenia patients; and 17 healthy control subjects. BOLD activation was assessed as a function of increasing task difficulty within and between groups as well as the main effects of cannabis use and the diagnosis of schizophrenia. Cannabis users and non-drug using first-episode schizophrenia patients exhibited equivalently reduced dorsolateral prefrontal activation in response to task difficulty. A trend towards additional prefrontal and left superior parietal cortical activation deficits was observed in cannabis-using first-episode schizophrenia patients while a history of cannabis use accounted for increased activation in the visual cortex. Cannabis users and schizophrenia patients fail to adequately activate the dorsolateral prefrontal cortex, thus pointing to a common working memory impairment which is particularly evident in cannabis-using first-episode schizophrenia patients. A history of heavy

cannabis use, on the other hand, accounted for increased primary visual processing, suggesting compensatory imagery processing of the task.

Cerebellar dysfunction has been proposed to lead to "cognitive dysmetria" in schizophrenia via the cortico-cerebellar-thalamic-cortical circuit, contributing to a range of cognitive and clinical symptoms of the disorder, including executive function deficits. In a subsequent study, cerebellar grey and white matter volumes and cerebellar regional grey matter abnormalities was measured in 13 remitted first-episode schizophrenia patients with less than two years' duration of illness. Patient data were compared to 13 pair-wise age, gender, and handedness-matched healthy volunteers using cortical pattern averaging on high-resolution magnetic resonance images. Total cerebellar volume and total grey matter volumes in first-episode schizophrenia patients did not differ from healthy control subjects, but total cerebellar white matter was increased and total grey to white matter ratios were reduced in patients. Four clusters of cerebellar grey matter reduction were identified: (i) in superior vermis; (ii) in the left lobuli VI; (iii) in right-inferior lobule IX, extending into left lobule IX; (iv) bilaterally in the areas of lobuli III, peduncle and left flocculus. Grey matter deficits were particularly prominent in right lobuli III and IX, left flocculus and bilateral pedunculi. These cerebellar areas have been implicated in attention control, emotional regulation, social functioning, initiation of smooth pursuit eye movements, eye-blink conditioning, language processing, verbal memory, executive function and the processing of spatial and emotional information. Consistent with common clinical, cognitive, and pathophysiological signs of established illness, the findings demonstrate cerebellar pathology as early as in first episode schizophrenia.

The contribution of cannabis use to schizophrenia neuropathology remains controversial. The cerebellum possesses a high density of cannabinoid type 1 receptors involved in the neuronal diversification of the developing brain. Cannabis abuse may interfere with this process during adolescent brain maturation leading to "schizophrenia-like" cerebellar pathology. Hence, magnetic resonance imaging

and cortical pattern matching techniques were used to investigate cerebellar grey and white matter in first-episode schizophrenia patients with and without a history of cannabis use and non-psychiatric cannabis users. In the latter group a lifetime dose dependent regional reduction of grey matter in the right cerebellar lobules was found and a tendency for more profound grey matter reduction in lobule III with younger age at onset of cannabis use. The overall regional grey matter differences in cannabis users were within the normal variability of grey matter distribution. By contrast, the previous study did demonstrate that first-episode schizophrenia subjects had lower total cerebellar grey to white matter ratios and marked grey matter loss in the vermis, pedunculi, flocculi and lobules when compared to pair-wise matched healthy control subjects. This pattern and degree of grey matter loss did not differ from age-matched first-episode schizophrenia subjects with co-morbid cannabis use. The findings indicate small dose-dependent effects of juvenile cannabis use on cerebellar neuropathology but no evidence of an additional effect of cannabis use on cerebellar grey matter pathology in first-episode schizophrenia.

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Cannabis has the lowest initiation age of any illicit drug, with onset of use typically occurring in early adolescence [1, 2]. An Australian household survey showed that a little over one-third of persons aged 14 years and over had used cannabis at some point in their lives, and 17% reported having used it within the past 12 months [3]. Recreational users seek its induction of euphoria, depersonalisation, somnolence, altered sensory perceptions and time sense, and relaxation [1, 4]. Acute intoxication can also produce less desirable perceptual, cognitive and motor impairments in healthy users [1, 4, 5] and exacerbate pre-existing disturbances in these domains in those diagnosed with schizophrenia [6, 7]. These findings suggest that cannabis use alters the functioning of brain regions responsible for control of cognition and maintenance of intact perceptual functions. Until recently, however, the physiological basis for these disturbances, the interaction between the constituents of cannabis and the endogenous cannabinoid system, were unknown. In this review we appraise the literature regarding cognitive, perceptual and neurobiological effects of cannabis in light of these recent developments, with the goal of integrating this evidence in the context of the links between cannabis and psychosis.

1.1. Cannabis and cognitive dysfunction

The acute and residual (within 12-24 h) neuropsychological effects of cannabis use include the induction of deficits in attention, executive functioning and short-term memory [8, 9]. Heavy cannabis use impairs processing speed and the ability to focus attention and ignore irrelevant information in long-term users, functions mediated by the prefrontal cortex (PFC) [10]. Solowij et al. found that these

[1] Cohen, M., Solowij, N., Carr, V. Cannabis, cannabinoids and schizophrenia: integration of evidence. *Australian New Zealand Journal of Psychiatry* **2008**, 42:357-368

deficits last beyond the period of intoxication and worsen with increasing years of cannabis use [10]. Other studies have also shown that there are longer term effects (24 h-28 days) of cannabis on short-term memory and attention that are dose dependent [11-15]. A meta-analysis of a small number of studies of cannabis users suggested that if any deficits exist in this population beyond the acute intoxication period, they are most likely to occur in the domain of learning and forgetting or retrieval of information [15].

1.2. Brain imaging in cannabis users

Several neuroimaging studies have explored the cerebral correlates of the acute and residual effects of cannabis use. When participants are at rest (i.e. not cognitively engaged in a task), dose related increases in global cerebral blood flow have been detected in intoxicated experienced users [16], with regionally specific changes in metabolism and cerebral blood flow in the orbitofrontal and PFC, basal ganglia [16, 17], insula, cingulate gyrus, and subcortical regions [18-20]. By contrast, recently abstinent experienced cannabis users show evidence of subnormal global cerebral blood flow [21, 22] and reduced cerebellar blood flow [18].

Tasks that are designed to engage specific cortical regions have been used in the imaging environment to assess the effects of cannabis on brain activation and function. Studies that have explored cerebral activity in acutely intoxicated participants have found in- creased regional cerebral blood flow (rCBF) in the anterior brain, predominantly in the paralimbic regions compared to healthy control subjects. These changes may underpin the mood-related effects of the drug [8, 23]. In contrast, decreased rCBF in the temporal lobe auditory area, visual cortex, and frontal regions was associated with impairment of attention [8, 23]. Kanayama et al. found that heavy long-term cannabis users abstinent for 6-36 h displayed greater and more widespread brain activation than non-using subjects while performing a spatial working memory task [21]. Persistent changes from expected patterns of cerebral activation have even been detected after 25 days

[13] and 28 days of abstinence [22]. Researchers have proposed that additional brain regions not usually involved in the task may be called upon to support the appropriate resolution of the cognitive challenge [21, 24]. However, it remains unclear whether these findings relate to cerebral adaptations to compensate for subtle residual effects of cannabis use or whether functional neuroimaging and neuropsychological testing evaluate different constructs altogether [see [25] for an in-depth review].

The changes in cerebral perfusion in cannabis users both while cognitively engaged and at rest are robust. But the evidence for structural brain change associated with cannabis use is equivocal. Positive findings include global reductions in cortical grey matter density in adolescent long-term cannabis users [26], generalized cerebral volume loss, smaller cerebellar vermi and focal temporal and frontal white matter abnormalities [27]. More convincing evidence of structural change has been demonstrated by the detection of grey and white matter density changes in heavy adult users [28] that included the same regions (e.g. parahippocampal gyrus) that showed altered activation in a decision-making task [22], and some of the density changes correlated with duration of cannabis use [28]. Most recently, a significant dose-related reduction of hippocampal and amygdala volumes has been reported in a small but well-controlled sample of very heavy long-term cannabis users [29], corroborating evidence from the animal literature. Interestingly, left hippocampal reduction was associated with subthreshold psychotic symptoms in the otherwise healthy cannabis users. More studies are required to clarify whether cannabis has direct neurotoxic effects altering brain structure. Newer enhanced anatomical data acquisition and brain structure analysis techniques [30] are likely to assist in this endeavour.

In summary, imaging studies demonstrate that cannabis use can result in regionally specific changes in the perfusion of frontal and memory-related cortical areas despite abstinence from cannabis and in the absence of detectable gross neuropsychological deficits. A compensatory cerebral mechanism, whereby

additional brain regions are recruited to account for subtle cognitive deficits [13, 21] is suggested. Although there is evidence of structural brain changes associated with cannabis use, a thorough interpretation of these findings cannot yet be made.

1.3. Links between cannabis use and psychosis

An association between cannabis use and psychotic symptoms and/or schizophrenia has been evident for some time [1]. Hambrecht and Häfner conducted a cluster analysis of early psychosis patients for whom cannabis use was linked to the onset of psychotic symptoms [31]. They proposed three potential types of association. First, in the absence of a known genetic liability to schizophrenia, cannabis use was proposed to reduce the 'schizophrenia vulnerability threshold', thereby increasing the susceptibility to psychosis. The Andreasson et al. 1987 study was the first to report a dose-response relationship between the amount of cannabis used in adolescence and the subsequent risk of developing schizophrenia [32]. More recent analyses of these data that controlled for personality traits, amphetamines and other drugs concluded that if cannabis could be removed from the 'schizophrenia equation', 13% of cases of schizophrenia might be prevented [33]. Van Os et al. also showed, in a well-controlled prospective study, that the extent of lifetime exposure to cannabis constituted an independent risk factor for the emergence of psychosis in a dose-response manner in previously psychosis-free persons [34]. Among users with no genetic liability to psychosis, cannabis was linked to the development of psychosis in up to 50% of cases. In the same year, Arsenault et al. reported that after controlling for pre-existing psychotic symptoms, cannabis use increased the risk of developing schizophrenia symptoms, that this risk was specific to cannabis as opposed to the use of other drugs and that early onset cannabis use (prior to age 15) conferred the greatest risk [35]. One possible explanation for the latter finding is that the transition from the cognitive, behavioural and emotional capacities of childhood to those of adulthood entails sensitive

neurodevelopmental processes, particularly of the frontal and limbic areas [36] and these maturational processes are particularly sensitive to the effects of drugs such as cannabis [37, 38].

The second type of association reported by Hambrecht and Häfner was that cannabis use was proposed to be the stress factor that precipitated the onset of psychosis in the context of a genetic predisposition to schizophrenia [31]. A number of studies provide support for this second grouping. Schizophrenia patients who use cannabis heavily have been found to be 10-fold more likely to have a family history of schizophrenia [39]. Moreover, a strong interaction between cannabis use and vulnerability to psychosis has been reported. Van Os et al. found that approximately 80% of psychosis outcome was attributable to the synergistic action of these two risk factors [34]. There may therefore be an interaction between a genetic risk for schizophrenia and cannabis acting as a biological stressor, triggering the onset of schizophrenia [40]. Recent work by Caspi et al. showed that a functional polymorphism in the catechol-O-methyltransferase (COMT) gene that results in less efficient breakdown of dopamine in the synapse by COMT, moderated the effect of adolescent cannabis use on the risk for adult psychosis [41]. Individuals homozygous for the COMT valine-158 allele, associated with increased dopamine levels in midbrain neurons that project to the ventral striatum [42], were at least fivefold more likely to develop symptoms of schizophrenia after adolescent exposure to cannabis than individuals homozygous for the methionine allele. It is biologically plausible that the cannabis users who are prone to psychosis may be unable to clear the mid-brain dopamine release known to be triggered by cannabis use [43]. The third type of proposed association was that of cannabis use as a form of self-medication to alleviate the dysphoria associated with the negative and depressive symptoms of schizophrenia. This has not, however, been borne out by the research [44]. An alternative proposition, the reverse causality model tested by Fergusson et al., asserts that the predominant direction of causality was from cannabis use to psychotic symptoms rather than the converse [45]. This suggests an explanation

for the associations between cannabis use and the onset of psychosis in those who are psychosis prone. But researchers have argued that if cannabis use had an aetiological role in the development of schizophrenia, then the incidence of schizophrenia should increase in parallel with increases in cannabis use, and they argue that it has not [5]. Recent research that tracked the incidence of schizophrenia and its relationship to cannabis use in South London between 1965 and 1999 showed that the number of cases of schizophrenia has doubled in this period [46] and that there was also a large increase in the proportion of schizophrenia patients who had used cannabis in the 12 months before diagnosis [47]. Using a conservative model that regards the heavy use of cannabis as one of several risk factors for schizophrenia, with a pooled odds ratio of 2.1 [44], Hickman et al. calculated that the drug would be responsible for 10% of new schizophrenia cases [48, 49].

1.4. Cannabis use in schizophrenia

Twenty-five per cent of schizophrenia patients meet lifetime criteria for a cannabis use disorder, situating it as the most commonly used illicit drug among this patient population [50]. Cannabis-using patients experience more psychotic symptoms [51], respond poorly to neuroleptic medications [52], have poorer treatment compliance and worse clinical outcomes; experience more relapses [5] and more hospitalizations [6, 53]. These observations are biologically plausible given that psychotic disorders involve disturbances in dopamine neurotransmitter systems [54] and cannabis increases dopamine release [55]. Paradoxically, cannabis may also have symptom-relieving effects [56], helping to ameliorate negative symptoms, depression, and the side-effects of antipsychotics, as well as relieving boredom, providing stimulation and facilitating socialization with peers, with patients reporting similar reasons for using cannabis as reported in the general population [57-59]. It is possible that the beneficial and adverse effects of cannabis might be dose related. Alternatively, the conflicting conclusions of self-report and epidemiologic studies may be reconciled by the possibility that

cannabis effects vary with time, so that schizophrenia patients may derive some short-term benefits from cannabis at the expense of adverse consequences in the longer term. A further explanation may be that brain regions associated with the positive, cognitive and negative symptoms of schizophrenia, which occur in various combinations from one patient to another, may respond differently to cannabis.

1.5. Endogenous cannabinoid system and changes in schizophrenia

Endocannabinoids are a family of lipid molecules involved in neuromodulation and neuroprotection [60-62]. Anandamide and 2-arachidonoyl-glycerol (2-AG) are produced on demand from membrane phospholipids [63] and once released are rapidly inactivated by membrane-bound fatty acid amide hydrolase expressed in cortical neurons [64]. Endocannabinoids are agonists at the central nervous system cannabinoid type 1 (CB1) receptor. These receptors are located presynaptically on inhibitory and excitatory neurons, where their activation causes transient neuronal suppression [65, 66]. This endocannabinoid-mediated 'retrograde signaling' has been reported to regulate both short- and long-term synaptic plasticity [67, 68]. Neocortical CB1 receptors are expressed mainly on g-aminobutyric acid (GABA) interneurons, while in the amygdala, hippocampus, basal ganglia and cerebellum they are expressed on both GABAergic and pyramidal neurons [64, 69, 70]. The location and function of the endogenous cannabinoid system suggest that it is positioned to regulate the neuronal circuits involved in cognitive function, emotions, and activity in the mesolimbic reward contingency pathways [71].

Changes in the endocannabinoid system have been reported to occur in schizophrenia. First, a triple repeat polymorphism of the CB1 receptor gene, located on chromosome 6q14-15 [72], a site that includes a schizophrenia susceptibility locus 6q13-q26 [73], has been associated with the hebephrenic type of schizophrenia [74]. Second, an increased expression of the CB1 receptor has

been found in the PFC in schizophrenia independent of recent exposure to cannabis [75], whereas increased CB1 density in the caudate and putamen was independent of diagnosis but correlated with recently ingested cannabis [76]. Increased CB1 densities have also been observed in the anterior and posterior cingulate in schizophrenia [77, 78]. The expression of endocannabinoids is also altered in schizophrenia [79], with a two-fold elevation in the concentration of cerebrospinal fluid (CSF) anandamide levels found in a small sample of schizophrenia patients [79]. In a replication study, an 8.5-fold elevation of CSF anandamide levels was found in a larger sample of neuroleptic-naıve schizophrenia patients, but there was no elevation of anandamide levels in a medicated patient sample, nor in a 'prodromal' group [80]. In non-medicated acute schizophrenia, CSF anandamide was negatively correlated with psychotic symptoms, which suggests that anandamide elevation in acute schizophrenia may reflect a compensatory adaptation to the disease state [81].

1.6. Cannabinoid effects within current hypotheses of schizophrenia

In the previous sections provided a selective overview of the effects of cannabis use in healthy individuals and in schizophrenia, the evidence for an association between cannabis use and an increased risk of schizophrenia and the changes to the endogenous cannabinoid system in schizophrenia. Next, the effects of cannabinoids in the context of prevailing hypotheses of schizophrenia will be explored.

1.7. Dopamine hypothesis

The schizophrenia syndrome comprises psychotic, negative and disorganisation symptoms, together with cognitive abnormalities. Dysfunction of dopamine neurotransmission in cortical and subcortical structures is central to the current understanding of schizophrenia. The dopamine hypothesis originally proposed that an excess of dopamine activity in the mesolimbic pathway between the

ventral tegmental area (VTA) and limbic structures caused psychotic symptoms [82]. Although psychosis is frequently the most striking clinical feature of schizophrenia, disturbances in mesocortical dopamine function associated with negative symptoms and cognitive deficits are now regarded as core features. The latter are thought to be related to reduced dopaminergic activity in the frontal lobes, particularly that mediated by D1 receptors [83].

1.8. Behavioural, biochemical, and electrophysiological

Data demonstrate the involvement of endogenous cannabinoids in regulating the activity of dopaminergic neurotransmission in the VTA and in the frontal cortex. The principal VTA neurons contain dopamine and regulate motivation, reward-related behaviours, salience attribution and cognition [84, 85]. The role of endogenous cannabinoids in the VTA has not yet been fully elucidated, but it is known that prolonged depolarisation of dopamine neurons within the VTA causes a transient calcium-dependent release of endocannabinoids. Anandamide and 2-AG serve as retrograde messengers [86] acting at presynaptic CB1 receptors localised on both excitatory and inhibitory neurons [87] and the release of 2-AG protects against excessive glutamate release, thereby reducing dopamine neuronal damage [88] during episodes of hypoxia or energy deprivation. Cannabinoids such as d-9-tetrahydrocannabinoland synthetic CB1 agonists increase the firing rate of mesolimbic dopaminergic neurons that terminate in the nucleus accumbens and PFC and enhance dopamine synthesis, release and turnover [55, 89, 90]. The mechanism that underpins cannabinoid-mediated mesolimbic dopamine agonism differs from that mediated by stimulants such as amphetamine or cocaine because cannabinoids do not act directly on mesolimbic dopamine neurons. Inhibition of GABA release from interneurons in the nucleus accumbens by exogenous cannabinoids acting at CB1 receptors (on those interneurons) and/or inhibition of glutamatergic pyramidal neurons that excite GABAergic inhibitory interneurons have been proposed as the mechanisms of

dopamine agonism by cannabinoids [91, 92]. Cannabis-mediated increases in mesolimbic dopaminergic activity could provide an explanation for the reports of an increase in the relative risk of experiencing psychotic symptoms in healthy individuals who use cannabis [1], and account for the increased sensitivity of schizophrenia patients to the psychotomimetic effects of cannabis [7]. The mesocortical dopamine projection, from the A10 cell group in the VTA to the PFC, is of critical importance for some PFC functions, such as the modulation of attention and working memory [93]. The PFC has a high density of CB1 receptors, and cannabinoids have been shown to modulate the dopaminergic neuronal inputs impinging on PFC neurons [94, 95]. Lower dopamine turnover in the frontal cortex is associated with poor attention in laboratory animals [96], and stimulation of cortical dopamine levels improves their performance [97]. Acute administration of cannabinoid agonists potently increases frontal cortical dopamine metabolism and release [98]. But repeated exposure to CB1 agonists produces an adaptive change that decreases dopamine release in the PFC, but not in other dopamine-rich areas such as the nucleus accumbens or dorsolateral striatum, thus resulting in a functional lesion of the cortical dopamine system [94]. These effects of CB1 agonists in the PFC could provide a mechanism for understanding how chronic use of cannabis can induce cognitive deficits and negative symptoms, but in low doses and if used infrequently may improve cognition (as reported by patients). Thus, in schizophrenia, chronic CB1 activation could exacerbate the effects of pre-existing alterations of dopamine function [99]; that is, decrease mesocortical dopaminergic transmission and reduce D1 receptor density [100], while augmenting mesolimbic dopamine neurotransmission, thereby triggering psychotic symptoms.

1.9. GABA hypothesis

A defect in GABA neuronal functioning has been proposed to contribute to the array of disturbances in cognitive functions observed in schizophrenia [101]. Evidence cited in favour of this hypothesis includes: changes in gamma band (40 Hz) synchronisation [102], which is a marker of GABA functioning; alterations in

markers of cortical GABA neurotransmission in post-mortem schizophrenia studies [103]; and decreased density of GABA interneurons in the cerebral cortex of schizophrenia patients that, in turn, correlates with reduced concentrations of the enzyme responsible for GABA synthesis (GAD67) [104].

GABA interneurons are critical for complex information processing, context representation and maintenance of working memory by the regulation of gating, a process by which pyramidal cell excitation is fine-tuned for efficient information processing and signal transmission to other cortical and subcortical regions. CB1 receptors are localised presynaptically on cortical and hippocampal GABAergic interneurons [105], specifically the cholecystokinin-expressing basket cells [69, 70]. Activation of CB1 receptors reduces GABA release [106], increasing excitatory outputs from pyramidal cells, disrupting the synchronisation of pyramidal cell activity [66] and inhibiting the formation of new synapses between hippocampal neurons [107].

Disruption to GABA function in the hippocampus provides a basis for understanding the effects of cannabis in interfering with memory consolidation, associative functions and normal gating mechanisms. Because basket cells form dense axo terminal plexuses on pyramidal neurons, they are critical for orchestrating pyramidal cell synchrony in the gamma frequency range [108]. Oscillations in this high-frequency range 'bind' perceptual stimulus features detected by the sensory cortices into coherent perceptions. The coupling of neocortical and hippocampal gamma oscillations binds representations associated with currently perceived an retrieved information [66]. Therefore, augmentation of cannabinoid receptor activation by an overactive endogenous cannabinoid system, or by the use of cannabis, could effectively 'remove the brakes' on signalling within and between regions critical for regulating memory and executive functions. Schizophrenia-associated GABA dysfunction may in part explain the particular sensitivity that schizophrenia patients have to cannabis [7], because the drug augments the deficits already present in this disorder.

1.10. Glutamate hypothesis

The current formulation of the glutamate hypothesis of schizophrenia proposes that hypofunctional corticolimbic and mesocortical N-methyl-d-aspartate (NMDA) glutamate receptors produce a decrease in the stimulation of inhibitory cortical GABA inter-neurons [109]. The evidence indicates that schizophrenia patients have altered glutamate metabolism [110] and altered NMDA subunit receptor gene expression [111]. Pharmacological, biochemical and animal model studies support this hypothesis [see [112] for review], and negative, positive and disorganisation symptoms that mimic schizophrenia can all be triggered by the administration of antagonists of the NMDA subtype of glutamate receptors (e.g. phencyclidine, ketamine, MK-801) [113].

CB1 receptor activation reduces excitatory glutamatergic neurotransmission from afferent terminals in the VTA, striatum [86], substantia nigra pars reticulata, the subthalamonigral pathway [114], and cerebellum [115]. Although CB1 receptors are ex- pressed on inhibitory terminals in the subcortex and cortex, expression on glutamatergic terminals is mostly limited to the subcortex. In the hippocampus, CB1 activation by exogenous cannabinoids disrupts long-term potentiation and long-term depression, and inhibits hippocampal glutamate release [7]. It has been hypothesized that some of the key symptoms of schizophrenia, such as delusions and hallucinations, could be due to altered hippocampal control of cognitive function and sensory gating [116]. Changes in hippocampal function observed in schizophrenia [see [117] for review] may therefore be augmented by the effects of cannabis on the glutamatergic system, and thus account for patients' vulnerability to the amnesic effects of this drug [7].

1.11. Neurodevelopmental hypothesis

Kraepelin argued that some cases of schizophrenia resulted from early cerebral insults, later manifesting as a maldevelopment of the brain [118]. The modern form of the neurodevelopmental hypothesis proposes an interaction between

genetic and environmental events during critical early periods in neural growth and development that negatively influence the way neurons are laid down, differentiated and then selectively culled by apoptosis [119]. Because cerebral development is highly dependent upon preceding processes, a disturbance at any one point may alter the developmental trajectory of the subsequent processes. In this way repeated subtle brain insults may accumulate over time that in later life, usually adolescence or early adulthood, may become expressed as schizophrenia.

Proponents of this hypothesis cite as evidence an increased frequency of perinatal complications in schizophrenia, the presence of minor physical anomalies, neurological, cognitive and behavioural dysfunction long before illness onset, and an absence of gliosis in post-mortem schizophrenia brains [120]. Reduced GABAergic neuron cell counts in the cortex, atypical cortical cellular architecture, reduced density of the mesocortical dopaminergic projections to the PFC and altered NMDA subunit expression, have all been cited to support this hypothesis [103, 111]. Finally, the course and outcome of schizophrenia is thought to be incompatible with a purely degenerative illness. For an in-depth review of this topic see [121].

The significance of endocannabinoid signaling for human brain development and the neurodevelopmental hypothesis is underscored by observations of cognitive, motor and social deficits that last into adulthood in the offspring of mothers who smoked marijuana during pregnancy [122, 123]. The Ottowa Prospective Perinatal study found moderate cognitive deficits in exposed children when they were 4 days old and again at 4 years. Exposures was associated with lower scores on executive function tasks affecting self-regulatory abilities such as response inhibition, and at age 13-16 years deficits in sustained attention were detected [124, 125]. At age 18-22 years functional magnetic resonance imaging was used to assess brain activation to a response inhibition task [126]. Response inhibition is a component of executive functioning, requiring the integrity of the dopaminergic systems that subserve the PFC and its connections to the rest of the brain. The authors found increased activity in the right inferior frontal gyrus and premotor

cortex and left lateral orbital frontal gyrus, along with decreased activity in the left cerebellum. They concluded that prenatal cannabis exposure affects neural systems involved in response inhibition regulation at least into young adulthood. No association has been reported, however, between maternal cannabis smoking during pregnancy and schizophrenia in the offspring.

The mechanisms that underpin the effects of prenatal cannabis exposure on brain development remain unclear, but clues have emerged from the study of human fetal neural tissue. Wang et al. found that the distribution of fetal CB1 receptor mRNA was distinct from the adult pattern, with a high expression level within regions of the hippocampus and the amygdala [127]. A considerably weaker signal was found in other cortical regions, cerebellum and basal ganglia, which also contrasts with the adult distribution. A subsequent study found that in utero cannabis exposure produced alterations in D2 gene expression in distinct neuronal populations (amygdala) of the human male foetal brain [128]. Although that study was confounded by prenatal exposure to alcohol and nicotine, other recent findings show that endocannabinoid signalling regulates the proliferation, migration, specification and survival of neural progenitors [129-131], dictates the phenotypic differentiation of basket (GABAergic) cell migration and integration [132] and controls the establishment of synaptic communication and remodelling [61, 133] during cerebral development.

Although limited in their generalizability to humans, animal model cannabinoid research has shown that disturbances of endocannabinoid system homeostasis may be of particular significance for our understanding of the neurodevelopmental hypothesis of schizophrenia. Prenatal cannabis exposure in laboratory animals increases CB1 receptor numbers and alters their functioning [134]. The CB1 receptor upregulation resulted in the demasculinisation of male animals, cerebral asymmetries, impaired hypo- thalamic pituitary functioning [135] and nociception [136], changes in nigrostriatal dopamine system function, and impaired PFC dopaminergic activity [137]. Cannabinoids administered to animal fetuses increased levels of the dopamine synthesis enzyme tyrosine hydroxylase mRNA,

the protein and its activity, and disrupted the temporal sequence of events during the development of this neurotransmitter system [138]. Prenatal treatment with cannabinoids resulted in abnormalities in motor activity [139], sociality [140], stress response [141] and cerebral reward mechanisms [142]. The effects of treatment were detected only once the animals were 40 days of age (i.e. adolescence) [134] and the onset of abnormalities was delayed in female animals and less intense than in male animals [143], in striking parallel to the gender differences seen in schizophrenia. Schneider and Koch [144] also found prepulse inhibition deficits, object recognition memory impairments, and anhedonia/avolition in laboratory animals exposed to cannabinoids during puberty, but not in adult rats. Because the prepulse inhibition deficits were reversible with the acute administration of haloperidol, a dopamine receptor antagonist, they concluded that pubertal exposure to cannabinoids could be a model for studying the development of schizophrenia.

1.12. Discussion

The literature review focused on the significance of cannabis-mediated perturbations of brain function for schizophrenia. This is because until recently, the expansion in our knowledge and understanding of the endogenous cannabinoid system in regulating brain development and function has not been applied to further this debate. Cannabis use is highly prevalent among young people and the association between cannabis and the risk of subsequently developing schizophrenia is consistent, dose dependent and higher if cannabis is used at an early age. There is now evidence demonstrating an association between increased rates of cannabis use and new cases of schizophrenia [46, 47, 49].

The cannabinergic system regulates the development of dopamine systems, the differentiation of GABA interneurons, and the processes that regulate synaptogenesis and neural pruning, as well as the control of short- and long-term plasticity [67, 68]. Early onset cannabis use may interfere with these

developmental processes, constituting a neurodevelopmental insult, and account for the association between age of onset of use and an increased risk of later developing schizophrenia. Cannabis augments mid-brain dopamine release, which is known to be associated with the induction of psychosis, and when used in higher doses cannabis suppresses PFC dopamine utilisation, resulting in cognitive dysfunction. Evidence suggests that some individuals are particularly prone to these adverse effects of cannabis due to a functional polymorphism of their COMT gene, which reduces their capacity to metabolise dopamine [41].

Cannabis use is a double-edged sword for patients with established schizophrenia. Low doses may actually improve frontal lobe functioning by acutely increasing blood flow to cortices concerned with cognition, mood and perception, and by increasing the availability and utilisation of dopamine. These short-term benefits may, however, come at a longer term cost, because continued use depresses cerebral flow to these areas and high doses functionally denervate the mesocortical pathway. The augmentation of mesolimbic dopamine by cannabis opposes the therapeutic effects of antipsychotic drugs, and predisposes to exacerbation of psychosis. Cannabis also enhances glutamatergic activity while suppressing GABA function. Thus the often-reported exacerbation of psychosis and frontal lobe impairment in schizophrenia patients who use cannabis may be attributable to these neurophysiological processes. The significance of the reported alterations to the endocannabinoid system in established schizophrenia remains unclear, but the production of 2-AG by striatal neurons has been found to protect dopamine neurons from excitotoxicity during periods of ischaemia and energy deprivation. This finding suggests that the endocannabinoid changes found in schizophrenia may be a neuroprotective response to an as yet unknown primary pathology.

1.13. Conclusion and study overview

The risk of psychosis is increased by approximately 40% in people who have used cannabis. There is a dose-response effect, leading to an increased risk of 50-200%

in the most frequent users. If having ever used cannabis increases risk of a psychotic outcome by 1.4-2.1-fold [44, 145] (as suggested by pooled analyses), then approximately 14% of psychotic outcomes in young people would not have occurred if cannabis had not been consumed. We do have evidence that cannabis use alters the normal development of the brain dopamine systems, shifts neurotransmitter physiology towards that seen in schizophrenia and reduces frontal and temporal lobe perfusion. By sharpening our research focus on these physiological perturbations and their relationship to the onset and exacerbation of schizophrenia, our understanding of the biological basis of the disorder is likely to be enhanced.

The current thesis will investigate some of the here reviewed aspects of cannabis use on brain function and structure in young people experiencing their first-episode of schizophrenia. Firstly, the effect of heavy cannabis use on executive function will be assessed with functional brain imaging in first-episode schizophrenia patients with and without a history of cannabis use and compared to young non-psychotic people with an equivalent history of cannabis use as well as to non-drug using healthy people. The focus will then shift to cannabis effects on brain pathology in cerebellum. The cerebellum plays an important role in cognition and has been identified to also play a critical role in schizophrenia psychopathology. Of particular interest is the high density of CB1 receptors in cerebellum, which may mediate the potential effects of cannabis abuse on impaired brain function and brain pathology as they occur in schizophrenia.

2. Functional magnetic resonance brain imaging of executive cognitive performance in young first-episode schizophrenia patients and age-matched long-term cannabis users[2]

2.1. Introduction

Cannabis is the most commonly used illicit drug and it is generally perceived as being relatively safe. One third of all Australians over the age of 14 years [146] and 40% of all Americans over the age of 12 years [147] have used it at least once. Recent evidence suggests that cannabis use is a risk factor for schizophrenia, produces cognitive dysfunction in healthy individuals, and exacerbates cognitive and positive symptoms in schizophrenia. Remarkably few brain imaging studies have examined the effects of frequent cannabis use on human brain function and to our knowledge, none have examined the effects in schizophrenia patients.

Acute effects of tetrahydrocannabinol (THC) on executive function are well documented [148-151]. Long-term frequent cannabis use may have specific detrimental effects on frontal lobe function. Deficits in the executive control of cognition and in the organisation and integration of complex information have been detected in recently abstinent cannabis users [11, 152, 153]. These findings are of concern as long-term users typically function in an unintoxicated but cognitively impaired state for substantial periods [154]. More controversial is the question as to whether long-term cannabis use can cause irreversible deficits in higher brain function that persist even after drug use stops. Two recent meta-analyses [15, 155] concluded that after prolonged abstinence, cannabis related cognitive dysfunction may be almost entirely reversible. However, imaging investigations of brain function

[2] Cohen, M., Johnston, P., Ehlkes, T., Fulham, R., Ward, P., Thienel, R., Rasser, P., Carr, V., Baker, A., Schall, U. Functional magnetic resonance brain imaging of executive cognitive performance in young first-episode schizophrenia patients and age-matched long-term cannabis users. Submitted to: *Psychiatry, Neurology & Brain Research*

in abstinent users have found that regardless of abstinence status, long-term cannabis exposure produces abnormalities in degree and location of brain activation [13, 22, 24]. For example, cannabis use alters the neural networks active to a visual-attention task in recently abstinent and currently active users who performed equally well on neuropsychological testing and on the task [24]. In abstinent users, hypoactivity in the left anterior cingulate and lateral prefrontal cortex, and hyperactivity in the hippocampus bilaterally on the Stroop task have been reported [13], whilst in 28 day abstainers, hyperactivity in the left cerebellum and hypoactivity in the right lateral orbitofrontal and dorsolateral prefrontal cortex were detected on a decision-making task (Iowa Gambling Task) [22]. It is unclear whether changes in brain activity detected in neuroimaging studies relate to subtle residual effects where brain activity has adapted to limit impairment or whether the functional neuroimaging and neuropsychological testing evaluate different constructs altogether [25].

There has been long-standing controversy as to whether heavy frequent cannabis use might precipitate a schizophrenia-like psychosis in people who had not previously shown psychotic symptoms. Four of five recent reviews [156-160] have concluded that adolescent cannabis use is a contributory cause of psychosis. The pooled odds ratio was 2.1 (95% CI: 1.7-2.5), which cannot be explained by confounding factors or reverse causality [160]. A gene-environment interaction, where a functional polymorphism in the catechol-O-methyltransferase (COMT) gene interacts with adolescent-onset cannabis use to predict the emergence of adult psychosis, may help to explain this association [41]. In established schizophrenia, cannabis use may exacerbate psychotic and cognitive symptoms, contribute to poor outcome and increase the likelihood of relapse [7, 161, 162]. There is a substantial literature linking impairments in executive control and abnormal activation in several regions of the prefrontal cortex in schizophrenia [163-167] and to dopamine modulation of executive function [168]. Therefore, cannabis may serve to augment pre-existing frontal lobe deficits in schizophrenia. However, the potential effects of cannabis use in adding to and/or unmasking impaired executive functioning in first-

episode schizophrenia have not been systematically investigated using functional brain imaging techniques.

The Tower of London task (TOL) task, an adaptation of the Tower of Hanoi, activates planning related brain regions by requiring subjects to calculate the number of moves necessary to shift a given configuration of coloured balls on pegs presented on a display screen to a goal configuration. TOL performance predominantly engages the prefrontal cortex [169-171]. Previous studies have demonstrated poor TOL performance in patients with frontal brain lesions [172-175], frontal lobe dementia [176], and schizophrenia [175, 177-179]. Dagher and colleagues [180] used this task in the Single Photon Emission Tomography (SPECT) environment to measure regional cerebral blood flow (rCBF) dependent activation to task complexity in healthy subjects, which was defined as the number of moves required to solve a TOL problem. They found that rCBF in the dorsolateral prefrontal cortex (dlPFC), lateral pre-motor cortex, rostral anterior cingulate cortex and dorsal caudate nucleus increased with task complexity. These findings have been confirmed by our group and others measuring the variation in blood oxygenation level dependent (BOLD) response as a function of task difficulty in the functional magnetic resonance imaging (fMRI) environment [179, 181, 182]. Dorsolateral prefrontal cortex task-difficulty dependent activation was confirmed when employing a regression model with number of moves required to solve a problem as the independent variable and BOLD signal changes as the dependent variable [179, 181, 182]. Additional activation related to task demands was reported for the parietal lobes (i.e., precunei and parietal lobules) while superior and middle temporal lobe BOLD contrasts inversely correlated with task demands. Rasser and colleagues [179] found reduced prefrontal cortical activation in first episode schizophrenia despite no significant differences from control subjects in behavioural performance. They also reported that reduced activation in the dorsolateral prefrontal cortex correlated with reduced cortical grey matter thickness in the same region.

The present study compared event-related BOLD responses to TOL task demands between young first-episode schizophrenia patients with and without heavy cannabis use and matched non-psychotic community volunteers with and without heavy cannabis use. It was predicted that there would be a reduction in activation of the TOL neural network in response to task demands (i.e., visual spatial working memory function; [179, 181]) in both first-episode schizophrenia patients and non-psychotic cannabis users, with more pronounced activation failure in these networks evident in first episode schizophrenia patients who were also heavy cannabis users.

2.2. Materials and methods

Ethics approval for this study was granted by the human research ethics committees of the University of Newcastle, the Hunter Area Health Service, and the University of Essen-Duisburg. After complete description of the study written informed consent was obtained from all participants.

2.2.1. Subjects

MRI data were collected from 53 subjects in four groups matched for gender, age and handedness. (1) Twelve first-episode schizophrenia patients diagnosed according to DSM-IV criteria, with less than two years duration of illness and no concurrent diagnosis of substance abuse. (2) Seventeen unintoxicated (not having used cannabis for a minimum of 24 hours prior to scanning session) age and gender-matched long-term cannabis users (minimum of 2,000 doses and duration of more than 2 years of use [15]. These subjects did not meet abuse or dependence criteria for alcohol or substances other than cannabis as measured by the Structured Clinical Interview for DSM [183], and had no personal or family history of psychiatric disorder. The Opiate Treatment Index [184] and a clinical assessment of patterns of cannabis use were used to calculate the quantity/frequency estimates of cannabis use. (3) Seven unintoxicated schizophrenia subjects with less than two years duration of illness and a minimum of a two-year cannabis-use history of at least 2,000 doses. (4) Seventeen age and gender-matched healthy subjects served as a control group. Cannabis using participants were required to

remain abstinent from cannabis use for twenty four hours prior to their imaging session. Exclusion criteria for all groups included a history of significant head injury (e.g., unconsciousness), relevant neurological (i.e., epilepsy) or medical (i.e., endocrine) conditions, claustrophobia or other anxiety disorder, and a failure to complete at least three years of high school. All schizophrenia subjects and cannabis users were assessed using the Structured Clinical Interview for DSM-IV Axis I Disorders (patient version) [183]. Schizophrenia participants were tested while in remission and clinically stable on atypical antipsychotic monotherapy. The majority of patients was treated with risperidone (range 0.5 to 4 mg per day) while three patients were on olanzapine (range 10 to 25 mg per day), two patients on amilsulpride (400 mg per day), and one patient on clozapine (800 mg per day). All other participants were screened with the Structured Clinical Interview for DSM-IV Axis I Disorders (non-patient version) [183]. A clinical assessment was made as to the frequency and duration of cannabis use. Handedness was assessed using the Edinburgh Handedness scale [185].

Table 1. Tower of London performance data and demographic and clinical characteristics of study participants

	Healthy Subjects (N=17)	Cannabis Users (N=17)	First-episode Schizophrenia Subjects (N=12)	Cannabis-using First-episode Schizophrenia Subjects (N=7)
Gender (male/female)	15/2	15/2	11/1	5/2
Handedness (right/left)	17/0	15/2	12/0	7/0
Mean age in years at testing (standard deviation)	20.9 (2.5)	21.8 (2.6)	19.2 (2.3)	21.1 (2.2)
Age of diagnosis of schizophrenia in years (standard deviation)	-	-	18.1 (0.5)	19.9 (0.2)
Age of onset of cannabis use in years (standard deviation)	-	14.8 (1.7)	-	15.4 (2.2)

Mean number of cones of lifetime cannabis use (range)	-	18,960 (3,000-62,415)	-	21,830 (4,760-54,750)
Tower of London %-correct performance (standard deviation)	76.6 (12.3)	79.6 (18.9)	82.5 (8.9)	83.6 (7.2)

2.2.2. Task and Procedure

While lying in the MR scanner, each subject was presented with TOL images, using mirrors in the head coil to view a back-projected video display (Figure 1). The upper half of the image contained a goal configuration of three different coloured balls on three pegs. The subject was asked to determine the minimum number of moves required to reach this goal configuration, given the distribution of balls in the lower half of the image. Problems involving 1 to 7 moves ('active') were presented along with a baseline condition in which the upper and lower halves of the image were identical ('zero move'). After viewing the images for 30 seconds, image acquisition ceased and the subject was asked to verbalise the required number of moves. Functional MRI data were obtained for 21 problems, presented in 3 consecutive sequences of 7 problems each. Every sequence commenced and ended with a 'zero move' problem and alternated between 'zero move' and 'active' problems (Figure 1 A).

All subjects were trained on a computerised version of the TOL task [178] prior to scanning in order to achieve a minimum of 80% correct responses.

2.2.3. MRI Acquisition, Processing and Analysis

MRI data were acquired using a Siemens Magnetom Vision 1.5 T MRI scanner (John Hunter Hospital, Newcastle) or a Siemens Sonata 1.5 T MRI scanner (University Clinic, Essen) with echoplanar imaging (EPI). Forty-seven subjects were tested in Australia and 6 subjects in Germany (3 first-episode schizophrenia patients and 3 pair-wise age and gender-matched healthy control subjects). The task and imaging parameters were validated for the two sites in two previous studies [179, 181].

For each subject, high-resolution structural MR data was collected with approximate dimensions of 164 x 256 x 256 with each voxel (volume elements) being approximately 1.0 x 1.0 x 1.0 mm using a 3D MPRAGE protocol with TR=9.7 msec, TE=4 msec, and flip angle=12 degrees. Subsequently, EPI images were collected whilst subjects performed the 7 TOL problems using a 64 by 64 matrix to acquire 16 axial slices covering the entire brain (TR=2.7 sec; TE=70 msec; flip angle=90 degrees; 6.25 x 6.25 x 8 mm in-plane resolution; each voxel being 6.25 x 6.25 x 8 mm) resulting in 70 volumes per run. Each of the three runs per subject was concatenated to produce a sequence of 210 image volumes.

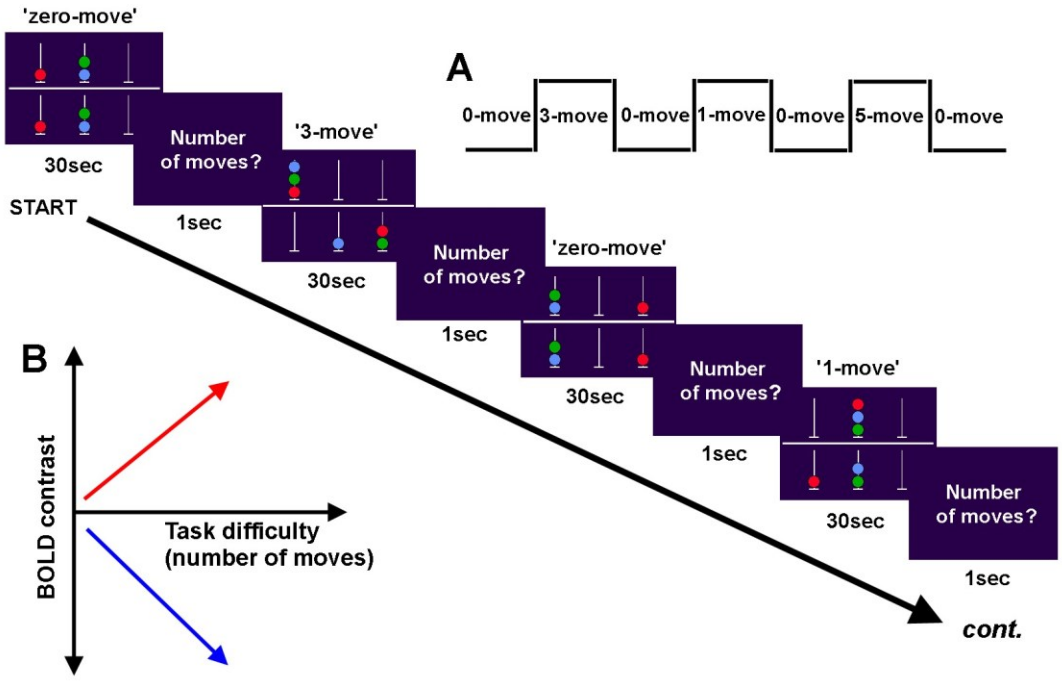


Figure 1: Subjects were presented with 21 Tower of London problems (presented in 3 consecutive sequences consisting of 7 problems) in the scanner using mirrors in the head coil to view a back-projected video display. The upper half of the image contained a goal configuration of three different coloured balls on three pegs. The subject was asked to determine the minimum number of moves required to reach this goal configuration, given the distribution of balls in the lower half of the image. Problems involving 1 to 7 moves ('active') were presented along with a baseline condition in which the upper and lower halves of the image were identical ('zero move'). After viewing each problem for 30 seconds, the subject was asked to verbalise the required number of moves. A: Example of a Tower of London presentation sequence. Each sequence commenced and ended with a

'zero move' problem and alternated between 'zero move' and 'active' problems. B: Statistical parametric maps of increasing (red) and decreasing (blue) Blood Oxygenation Level Dependent (BOLD) response that significantly correlated with Tower of London task difficulty (i.e. number of moves), served as the dependent functional imaging measure of the experiment [181]

Data were pre-processed and analysed according to a two-stage random effects model using SPM2 software [186]. Data for each subject were motion corrected and unwarped to reduce motion-induced artefacts. Motion corrected data were co-registered against the individual's high resolution structural volume, spatially smoothed using a Gaussian filter with a FWHM of 10 x 10 x 10 mm, and subsequently normalised into Montreal Neurological Institute (MNI) space [187] using the SPM2 T1 template image as the target volume. Data were high pass filtered with a cut-off of 128 sec.

Potential confounding effects due to scanner site were assessed by non-parametric permutation testing in order to address asymmetries of group compositions (i.e. 47 versus 6) using the SnPM toolbox [188]. Pseudo-t statistics were calculated for 10,000 permutations of labelling across the 47 contrast statistic images from the Newcastle site and 6 contrast statistic images from the Essen site.

BOLD signal changes were tested as a linear function of increasing task difficulty (i.e. number of moves; [181]) for all subjects and for each of the four subject groups, respectively. The statistical threshold was set to $P<0.05$ (corrected for multiple comparisons) while a more liberal $P<0.001$ statistical threshold (uncorrected for multiple comparisons) was employed for cannabis using first-episode schizophrenia patients in order to account for a smaller group size.

This was followed by a two-factor analysis of variance (ANOVA) of task difficulty-dependent BOLD response with factors (1) 'diagnosis of first-episode schizophrenia' [0=no/1=yes] and (2) 'cannabis use status' [0=no/1=yes] at $P<0.001$ (uncorrected for multiple comparisons) and reported for clusters of $\geq$ 10 consecutive voxels. This analysis was repeated with data collection site as covariate and at a more liberal $P<0.005$

statistical threshold (uncorrected for multiple comparisons), respectively. Subsequently, between-group comparisons (random effects model) were performed at $P<0.001$ (uncorrected for multiple comparisons) and reported for clusters of $\geq$ 10 consecutive voxels.

2.3. Results

Participant groups did not differ significantly in age, gender composition, handedness or age at diagnosis of schizophrenia. All participants performed the TOL task at an equivalent performance level of ≈80% correct responses whilst in the scanner (Table 1).

Significant BOLD signal increase (i.e. *positive* BOLD) as a linear function of increasing task difficulty (i.e. number of moves of the TOL task) was confirmed for left middle frontal gyrus (Brodmann Area [BA] 6), left superior parietal lobule (BA7) and cerebellum (left tonsil, left pyramis, and right tuber) whereas significant BOLD signal decrease (i.e. *negative* BOLD) was confirmed for left and right superior frontal gyri (BA8), left inferior frontal gyrus (BA47), right insula (BA13) and right postcentral gyrus (BA3; Table 2 and Figure 2). There was no evidence for site-related differences in activation levels during TOL performance. The correct labelling produced a maximum pseudo-t of 3.5 for permutation testing (10,000 iterations), which was close to the mean of the maximally observed pseudo-t scores across the permutation distribution (the threshold for statistical significance at $P<0.05$ for this permutation distribution was a pseudo-$t > 4.7$).

Table 2. Significant[a] Blood Oxygenation Level Dependent (BOLD) signal increase (i.e. positive BOLD) and significant BOLD signal decrease (i.e. *negative BOLD*) as a linear function of increasing task difficulty (i.e. number of moves of the Tower of London task) for all 53 study participants

Anatomical Region	Brodmann Area (BA)	Cluster Voxel Count	Talairach Coordinates x;y;z (mm) for Cluster Peak Activation	T_{max}	Z-Score
Positive BOLD					
Left Superior Parietal Lobule	7	4861	-28 -62 50	12.90	7.82
Left Middle Frontal Gyrus	6	7427	-30 -4 60	11.69	7.50
Right Cerebellum, Tuber		892	38 -64 -26	9.61	7.26
Left Cerebellum, Tonsil		1918	-36 -60 -32	9.16	7.04
Left Cerebellum, Pyramis		509	-4 -78 -26	6.79	5.72
Negative BOLD					
Right Superior Frontal Gyrus	*8*	*2860*	*14 38 54*	*11.87*	*7.75*
Left Superior Frontal Gyrus	*8*	*13738*	*-54 -12 0*	*11.45*	*7.76*
Right Insula	*13*	*5388*	*50 -32 20*	*9.06*	*6.99*
Right Postcentral Gyrus	*3*	*44*	*48 -18 56*	*6.16*	*5.32*
Left Inferior Frontal Gyrus	*47*	*16*	*-46 30 2*	*5.13*	*4.59*

a) Positive and negative BOLD contrasts statistically thresholded at $P<0.05$ with $\geq$ 10 consecutive voxels and corrected for multiple comparisons

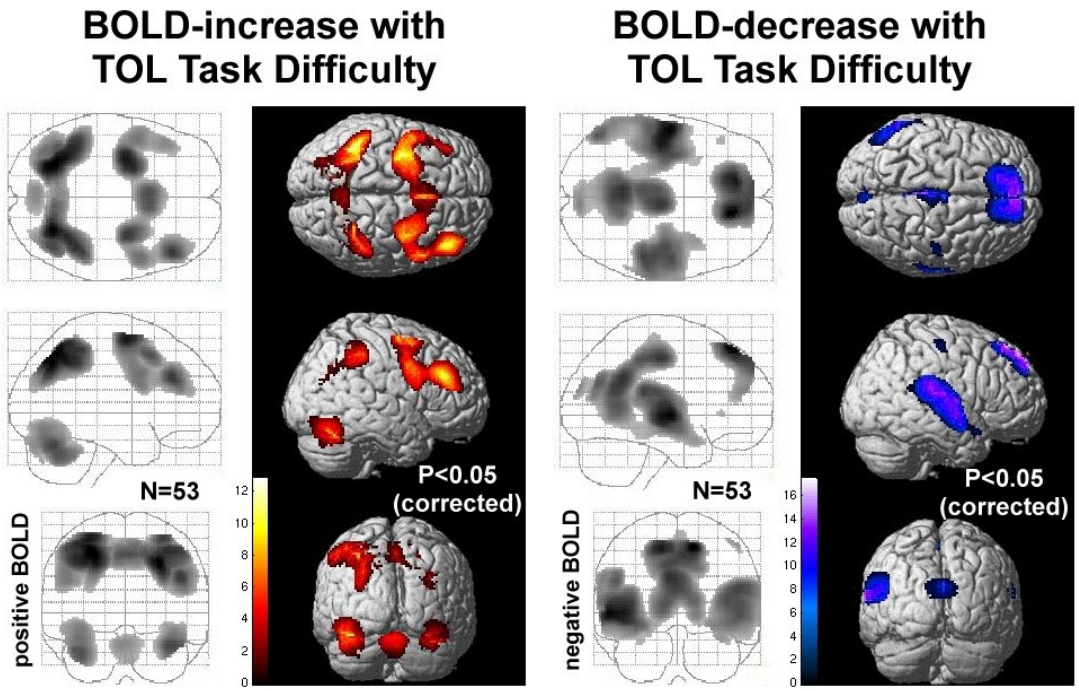


Figure 2: Glasshouse and brain surface projected (superior, right-lateral and posterior view) brain regions with significant[a] Blood Oxygenation Level Dependent (BOLD) signal increase (i.e. *positive* frontal, parietal and cerebellar BOLD) and significant BOLD signal decrease (i.e. *negative* prefrontal, frontal and temporal BOLD) as a linear function of increasing task difficulty (i.e. number of moves of the Tower of London task) for all 53 study participants. See Table 2 for detailed description of anatomical and statistical results

In healthy subjects, significant clusters of BOLD activation in response to increasing TOL task difficulty (i.e. number of moves) were identified in: the right inferior and middle frontal gyri (BA9 and BA6, respectively); the precuneus, the right superior and the left inferior parietal lobules (BA7 and BA40, respectively); and the right cerebellar tonsils and tuber (Figure 3 and Table 3). Clusters of *decreasing* BOLD activation with increasing task difficulty were confirmed for the left superior frontal gyrus (BA9) and the left middle and superior temporal gyri (BA21 and BA22, respectively).

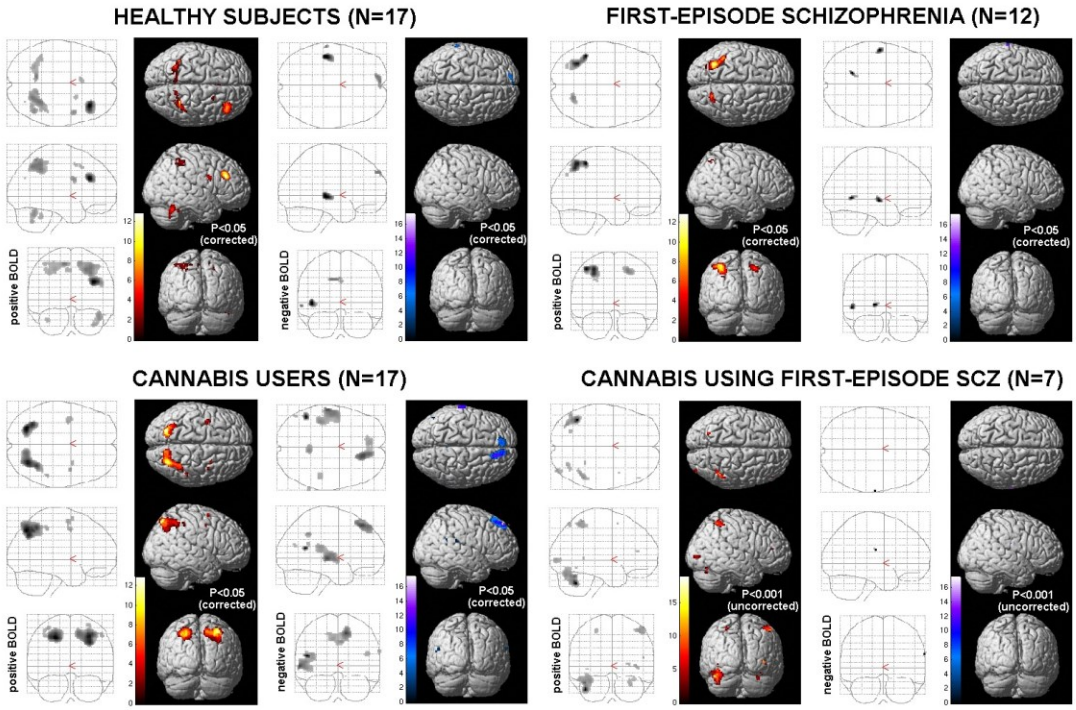


Figure 3: Glasshouse and brain surface projected (superior, right-lateral and posterior view) brain regions in healthy subjects, cannabis users and cannabis-using and non-using first-episode schizophrenia (SCZ) subjects, respectively, with significant clusters of positive and negative Blood Oxygenation Level Dependent (BOLD) contrasts[a] in response to task difficulty of the Tower of London task (i.e. number of moves). When healthy subjects are performing the task, right dorsolateral prefrontal, parietal (i.e. left and right precuneus), and right cerebellar (i.e. tuber) activation significantly increases (= positive BOLD) with task demands while left medial prefrontal and left middle temporal gyrus activation significantly decreases (= negative BOLD) with task difficulty. Note that first-episode schizophrenia patients and heavy cannabis users fail to activate the right dorsolateral prefrontal cortex when performing the task. See Table 3 for detailed description of further anatomical and statistical results

Table 3: Brain regions in control subjects, cannabis users and cannabis-using and non-using first-episode schizophrenia subjects, respectively, with significant clusters of positive[a] and *negative*[b] Blood Oxygenation Level Dependent (BOLD) contrasts in response to task difficulty of the Tower of London task

Anatomical Region	Brodmann Area (BA)	Cluster Voxel Count	Talairach Coordinates x;y;z (mm) for Cluster Peak Activation	T_{max}	Z-Score
HEALTHY SUBJECTS[c]:					
Right Middle Frontal Gyrus	9	351	36 30 28	12.8	6.1
Right Inferior Frontal Gyrus	9	52	46 4 28	8.1	5.0
Left Medial Frontal Gyrus	6	29	-6 16 46	7.2	4.7
Right Superior Frontal Gyrus	6	16	22 10 50	7.0	4.7
Right Precuneus	7	591	24 -60 48	8.9	5.3
Left Precuneus	7; 40	292	-10 -54 56	9.0	5.3
Left Inferior Parietal Lobule	40		-34 -52 44	7.2	4.7
Right Cerebellum, Tuber		139	44 -56 -26	8.2	5.1
Right Cerebellar Tonsil		23	-38 -46 -32	7.1	4.7
Right Cerebellum, Culmen		17	42 -48 -30	7.0	4.7
Left Superior Frontal Gyrus	*9*	*52*	*-14 58 36*	*-7.2*	*-4.7*
Left Temporal Gyrus	*22*	*142*	*-46 -24 -2*	*-8.2*	*-5.1*
Left Middle Temporal Gyrus	*21*	*11*	*-62 -24 -6*	*-7.2*	*-4.7*
CANNABIS USERS[c]:					
Left Middle Frontal Gyrus	6	51	-36 -4 60	7.2	4.7

Right Middle Frontal Gyrus	6	11	40 0 46	7.4	4.8
Right Superior Parietal Lobule and Precuneus	7; 17; 19	1171	30 -68 46	9.7	5.5
Left Superior Parietal Lobule	7	625	-24 -70 46	10.9	5.6
Right Inferior Parietal Lobule	40	14	50 -38 50	7.1	4.7
Right Superior Frontal Gyrus	*8*	*577*	*14 36 52*	*-9.9*	*-5.5*
Left Superior Temporal Gyrus	*22*	*865*	*-60 -18 0*	*-9.8*	*-5.5*
Left Superior Temporal Gyrus	*22*	*57*	*-48 -54 16*	*-12.1*	*-6.0*
Right Superior Temporal Gyrus	*41*	*40*	*48 -32 18*	*-7.2*	*-4.7*
Right Supramarginal Gyrus	*40*	*11*	*66 -48 22*	*-7.0*	*-4.7*
Right Precuneus	*31*	*70*	*6 -48 38*	*-8.2*	*-5.1*
FIRST-EPISODE SCHIZOPHRENIA SUBJECTS[c]:					
Left Inferior and Superior Parietal Lobule	7; 40	462	-44 -46 54	17.5	6.0
Right Superior Parietal Lobule	7	116	20 -66 52	11.6	5.2
Left Superior Temporal Gyrus	*22*	*46*	*-54 -10 -2*	*-12.7*	*-5.4*
Left Lingual Gyrus	*18*	*25*	*-18 -54 2*	*-12.9*	*-5.4*
CANNABIS-USING FIRST-EPISODE SCHIZOPHRENIA SUBJECTS[d]:					
Right Inferior Parietal Lobule	40	98	50 -48 62	9.1	3.9
Left Superior Parietal Lobule	7	19	-24 -68 58	6.2	3.4

Right Middle Occipital Gyrus	18	27	38 -90 2	7.4	3.6
Left Cerebellar Tonsils, Tuber and Pyramis		405	-42 -58 -36	18.8	4.8
Right Cerebellar Tuber and Declive		40	30 -74 -30	6.8	3.5

a) BOLD response that significantly increased with Tower of London task difficulty (i.e. number of moves) b) BOLD response *(in italic)* that significantly decreased with Tower of London task difficulty (i.e. number of moves) c) Data were statistically thresholded at $P<0.05$, corrected for multiple comparisons and reported for clusters of $\geq$ 10 consecutive voxels above threshold d) Data were statistically thresholded at $P<0.001$, corrected for multiple comparisons and reported for clusters of $\geq$ 10 consecutive voxels above threshold

In the cannabis-using non-psychotic group, a largely similar pattern of task difficulty-dependent bilateral parietal (BA7, 17, 19 and 40) and bilateral middle frontal gyri (BA6) activation was confirmed (Figure 3 and Table 3). In addition, decreased superior temporal gyral BOLD response predominantly in BA22 (left hemisphere) and, to some extent, in BA40 and 41 (right hemisphere) were found with increasing task demands. Right superior frontal gyrus (BA8) and right precuneus (BA31) activation was inversely correlated with task difficulty while dorsolateral prefrontal and cerebellar activation did not reach statistical significance in this group.

Significant task difficulty-dependent BOLD activation was also confirmed in the left inferior parietal and bilateral superior parietal lobules (BA7 and 40) in first-episode, non-using schizophrenia subjects. Dorsolateral prefrontal and cerebellar BOLD responses did not reach statistical significance, while left lingual (BA18) and left superior temporal gyri (BA22) activation decreased significantly with increasing task demands (Figure 3 and Table 3). Data from the small sample of cannabis-using, first-episode schizophrenia group ($P<0.001$ uncorrected for multiple comparisons) suggested a similar pattern of BOLD response with respect to parietal activation (Figure 3 and Table 3) and some right-occipital activation in BA18. Left cerebellar activation, however, appeared to be dominating in this group while decreasing BOLD activation with increasing task demands was not found anywhere. Even at this less conservative statistical criterion,

dorsolateral prefrontal activation did not reach statistical significance in this co-morbid group.

When assessing main effects for first-episode schizophrenia and cannabis use in the combined groups (ANOVA) increased BA46 and decreased BA8 activation in the right middle frontal gyrus was significantly associated with the diagnosis of schizophrenia while cannabis use was associated with an increase of BA18 activation in the left middle occipital gyrus (Figure 4). These main effects were confirmed when entering data collection site as covariate.

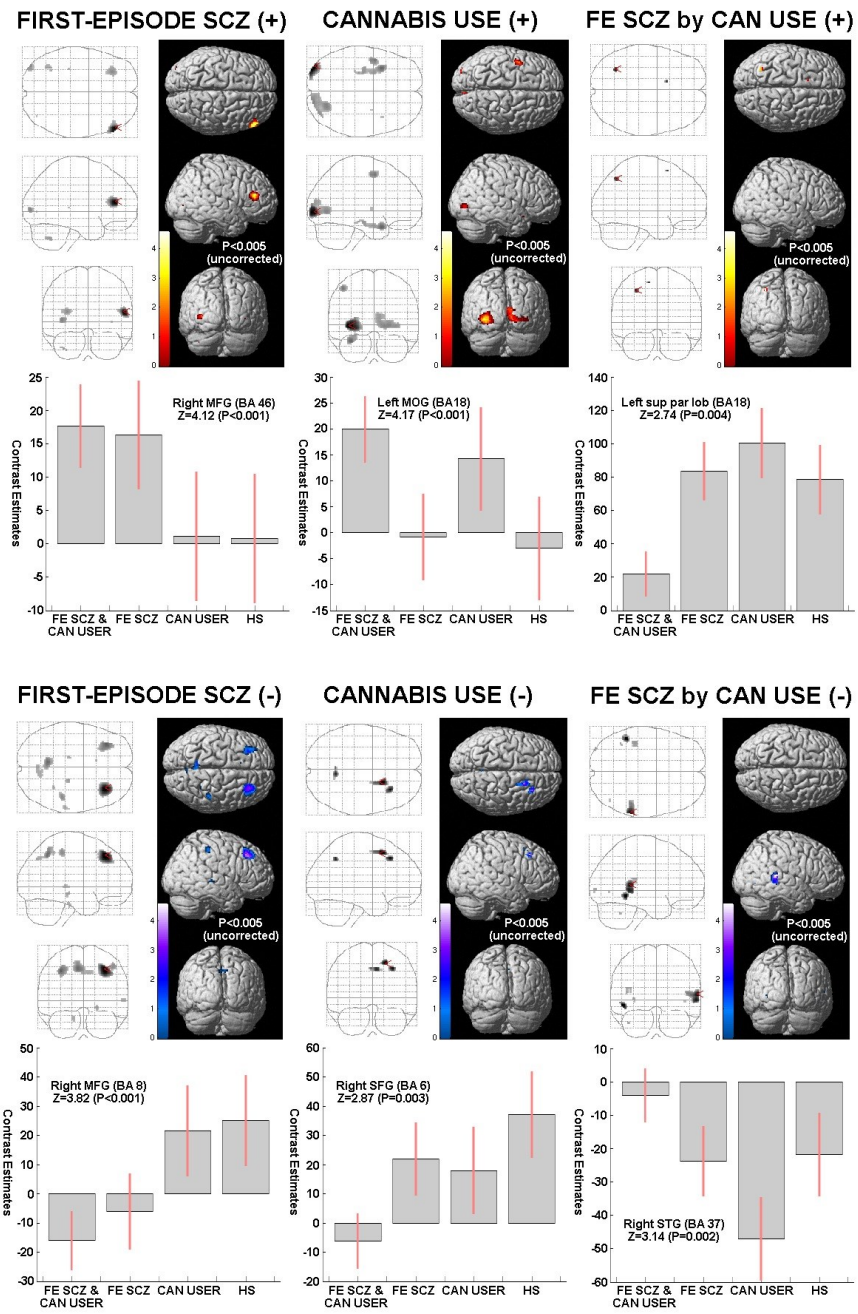


Figure 4: Glasshouse and brain surface projected regions (superior, right-lateral and posterior view) of increased (+) or decreased (-) Blood Oxygenation Level Dependent (BOLD) contrasts[a] derived from a two-factorial analysis of variance of cannabis (Can) use by diagnosis of first-episode (FE) schizophrenia (SCZ). Examples are provided for the right superior and middle frontal gyrus (SFG and MFG, respectively), the right superior temporal gyrus (STG), the left superior parietal lobule (sup par lob), and the left middle occipital gyrus (MOG). Brodmann areas (BA) are indicated. Histograms represent contrast estimates relative to baseline with standard deviation (HS = healthy subjects). The diagnosis of first-episode schizophrenia is significantly[b] associated with an increased BA46

and decreased BA8 BOLD activation in the right MFG while heavy cannabis use leads to a relative increase of BA18 BOLD activation in the left MOG. See Table 4 for detailed description of anatomical and statistical results

When applying a more liberal P<0.005 threshold (with ≥ 10 consecutive voxels and uncorrected for multiple comparisons; Table 4), diagnosis of first-episode schizophrenia also accounted for an increase of BOLD response in the left middle frontal gyrus (BA10), the left middle occipital gyrus (BA18), and the left cerebellar tonsil. Other areas included bilateral precunei (BA7), the right inferior parietal lobule (BA40), the parahippocampal gyrus (BA35), the right middle temporal gyrus (BA21), and the left pons. Cannabis use accounted for an increase of BOLD response in the left superior temporal gyrus, extending into the parahippocampal gyrus and amygdala (BA38), and the left middle frontal gyrus (BA6) while decreased BOLD response was found in the right superior and middle frontal gyri (BA6 and BA8, respectively) and the right precuneus (BA7) when applying the more liberal P<0.005 statistical threshold with ≥ 10 consecutive voxels (uncorrected for multiple comparisons). This analysis also suggests bilaterally differential BOLD activation in the temporal lobes (BA21, 22, 37 and 41, respectively) when analysing diagnosis of schizophrenia by cannabis use interactions. These interaction effects, however, were not confirmed after entering data collection site as covariate.

Table 4: Two-factorial analysis of variance of brain regions with increased or *decreased* Blood Oxygenation Level Dependent (BOLD) contrasts[a] related to cannabis use, the diagnosis of first-episode schizophrenia, and the interaction of cannabis use by diagnosis of schizophrenia, respectively

Anatomical Region	Brodmann Area (BA)	Cluster Voxel Count	Talairach Coordinates x;y;z (mm) for Cluster Peak Activation	T_{max}	Z-Score
CANNABIS USE:					
Left Middle Frontal Gyrus	6	75	-46 2 54	3.3	3.4
Left Superior Temporal Gyrus, Hippocampus (Parahippocampal Gyrus), and Amygdala	38	181	-34 12 -24	3.5	3.3

Right Lingual Gyrus and Right Inferior Occipital Gyrus	18	569	22 -70 0	3.2	3.1
Left Middle Occipital Gyrus	17	284	-36 -88 -2	4.6	4.2
Right Superior Frontal Gyrus	*6*	*83*	*20 14 52*	*-3.0*	*-2.9*
Right Middle Frontal Gyrus	*8*	*47*	*30 26 44*	*-3.0*	*-2.8*
Right Precuneus	*7*	*43*	*6 -56 42*	*-2.9*	*-2.8*
DIAGNOSIS OF FIRST-EPISODE SCHIZOPHRENIA:					
Right Middle Frontal Gyrus	46	151	54 40 14	4.5	4.1
Left Middle Frontal Gyrus	10	113	-30 34 16	3.1	3.0
Left Middle Occipital Gyrus	18	35	-38 -88 2	3.2	3.0
Left Cerebellar Tonsil		11	-36 -58 -40	3.0	2.8
Right Middle Frontal Gyrus	*8*	*405*	*32 32 46*	*-4.1*	*-3.8*
Left Middle Frontal Gyrus	*8; 9*	*183*	*-34 34 44*	*-3.4*	*-3.2*
Right Middle Temporal Gyrus	*21*	*14*	*60 -28 0*	*-2.8*	*-2.7*
Left Precuneus	*7*	*175*	*-8 -54 48*	*-3.3*	*-3.1*
Left Precuneus	*7*	*48*	*0 -70 42*	*-2.9*	*-2.7*
Right Inferior Parietal Lobule	*40*	*52*	*44 -32 50*	*-3.0*	*-2.9*
Right Parahippocampal Gyrus	*35*	*14*	*20 -22 -10*	*-2.9*	*-2.8*
Right Precuneus	*7*	*10*	*30 -46 52*	*-2.8*	*-2.7*
Left Pons		*11*	*-14 -22 -26*	*-2.8*	*-2.7*
INTERACTION OF CANNABIS USE BY DIAGNOSIS OF SCHIZOPHRENIA:					
Left Temporal Lobe	37	43	-50 -46 -8	3.4	3.2
Left Superior Temporal Gyrus	41	40	-42 -32 18	2.8	2.7

Right Superior and Middle Temporal Gyrus	21; 22; 41	191	62 -38 8	3.3	3.1
Left Superior Parietal Lobule	*7*	*12*	*-30 -62 48*	*-2.9*	*-2.7*

a) Positive and *negative (in italic)* BOLD contrasts statistically thresholded at P<0.005 with ≥ 10 consecutive voxels and uncorrected for multiple comparisons

Finally, group differences were tested using a random effects model and reported here at P<0.001 (≥ 10 consecutive voxels, ≥ Z=3.4, and uncorrected for multiple comparisons). Owing to the small sample size, the first-episode, cannabis-using schizophrenia patients were not included in this between-group analysis.

When comparing healthy control subjects with cannabis users, left insular (BA13) BOLD activation discriminated between the two groups. Contralateral middle temporal (BA21) and right middle frontal (BA8) BOLD activation discriminated between healthy control and first-episode, non-using schizophrenia subjects while BOLD activation in the left middle frontal gyrus (BA10) discriminated these first-episode schizophrenia subjects from cannabis users. This analysis confirmed differential right prefrontal activation when comparing schizophrenia and healthy control subjects whereas differential left prefrontal activation discriminated between non-psychotic cannabis users and schizophrenia subjects.

2.4. Discussion

The results confirm previous investigations that have reported activation of the right dlPFC (BA 9 and 46), and bilateral premotor area (BA 6), parietal lobes and cerebellum to correlate with task difficulty [180-182]. We predicted a reduction of task difficulty-dependent BOLD response in this network in both first-episode schizophrenia patients and non-psychotic cannabis users, with more pronounced reduction of BOLD response in these areas evident in first episode schizophrenia patients who were also heavy cannabis users. Our findings suggest similar alterations in brain activation to increasing TOL task difficulty in non-psychotic cannabis users and non-using first-episode schizophrenia

patients. There was also a statistical trend for cannabis-using first-episode schizophrenia subjects to show reduced BOLD activation in the left superior parietal lobule and a failure to decrease BOLD activation in the right medial superior temporal lobe with increasing task demands. While the diagnosis of schizophrenia largely accounted for the prefrontal deficit, cannabis use was predominantly associated with a task difficulty-related increase of BOLD activation in the visual cortex.

The individual findings will be subsequently discussed for each brain region, showing a differential BOLD response for diagnosis of first-episode schizophrenia and/or cannabis use history.

The within group analyses showed that first-episode schizophrenia was not associated with right dlPFC or premotor cortex positive BOLD activation to task difficulty as was found in control subjects. In schizophrenia, this is a frequent finding both at baseline e.g. [189] and during cognitive tasks that are designed to engage the prefrontal cortex (see [190] for review). As there were no significant differences in behavioural performance on the TOL task across participant groups, bilateral activation of the parietal lobules may have compensated for the deficit in prefrontal activation [179, 181].

The non-psychotic cannabis users did not show activation of the dlPFC to task difficulty. The present finding of deficient right dlPFC in cannabis users has previously been reported [13, 17, 191]. Based on these and past findings, the right DLPFC is thought to be part of a prefrontal neural network that is significantly affected by drug abuse [22]. In the TOL task, the right dlPFC is active when the demands on planning for a task increase such that additional manipulation or monitoring of information is necessary to support the integration and/or maintenance of stored representations in relation to a goal (i.e., executive processes) [192-196]. Newman and colleagues [196] have also shown right dlPFC activation to be highly correlated with individual differences in working memory, suggesting a potential common locus of dysfunction that may underpin the working memory deficits reported for both schizophrenia and cannabis use.

Cannabis users did activate the premotor cortex to task difficulty. Recent investigations have shown that this region is implicated in cognitive tasks such as mental arithmetic [197], spatial and non-spatial working memory [173], attention control [198], and mental imagery [199] as well as motor control. As such, this area may have assumed the role of task difficulty-based processing, suggesting neuroadaptation to long-term cannabis use [24]. Our finding of cerebellar hypoactivity is in agreement with the finding that chronic cannabis users show decreased cerebellar metabolism, which may be related to the effects of cannabis use on cerebellar cannabinoid binding sites [17].

Schizophrenia, cannabis use and the presence of both diagnoses were associated with common left superior parietal lobule positive BOLD activation. Newman and colleagues [196] showed that the parietal and prefrontal TOL networks work co-operatively to solve problems, with the right prefrontal and left superior parietal cortex being most active to task difficulty. They hypothesised that as imagery plays an important role in spatial reasoning and memory [200], which are not dissociable in this task, left parietal activation to task difficulty may indicate that this region serves as a visuo-spatial workplace for the (right) prefrontal cortex as TOL problem complexity increases. Therefore, we interpret this shared locus of differential activation to suggest a potential common cortical reserve to compensate for deficient and/or dysfunctional right dlPFC activity. That is, this area may have assumed the role at the 'top' of the processing network, supporting task difficulty-dependent processing.

There was an inverse relationship between BOLD signal and task difficulty for schizophrenia, cannabis users and healthy control subjects in the left temporal lobe. This pattern of decreasing temporal lobe BOLD activation to increasing task difficulty has been proposed to represent a down-regulation of activity in primary auditory information processing regions whereby competing non-task-relevant auditory sensory information is inhibited, thereby supporting attention to the task [179-181]. The cannabis using, first episode schizophrenia group did not show this

inverse relationship; however, interpretation of this finding is limited by the small subject numbers in this group.

The analyses for main effect of diagnosis of schizophrenia, which assessed discriminating cortical activation by diagnosis, showed prominent right dlPFC (BA 46) activation. One possible explanation, suggested by Callicott and colleagues [201], is that individuals with schizophrenia have a different relationship between task load and dlPFC activity (i.e., the load-response curve) compared to healthy subjects. At lower working memory loads dlPFC activity may be increased, but at higher working memory loads it is decreased (referred to as inefficient dlPFC activity; [176, 201]. Therefore, overall dlPFC activation would have been greater to TOL problems of low and moderate difficulty [179], reflecting that area's particular load-response curve, as represented in the main effect of diagnosis found in the present study. The schizophrenia patients also showed negative BOLD activation in the right frontal eye field (BA8) as a main effect of diagnosis. This region is active during the guiding of eye movements in the systematic intentional exploration of space [202]. A downgrading of visual search to facilitate the working memory processing of the demands of the task may explain this finding. As the present study design did not incorporate eye movement measurements, this interpretation would require further investigation.

The analysis for main effect of diagnosis showed that cannabis users activated the left middle occipital gyrus (V2). Numerous neuroimaging studies have shown that visual imagery, like visual perception, evokes activation in occipitoparietal and occipitotemporal visual association areas [203-205]. Furthermore, it appears that visual imagery evokes stronger responses in the left visual cortex, whereas visual perception evokes stronger responses on the right [206]. The primary visual cortex (V1) has direct reciprocal connections with posterior extrastriate areas including V2 [207, 208], which have reciprocal connections with the lateral intraparietal area, the frontal eye fields and the dlPFC. Thus, whilst the left superior parietal lobule may have been active to task difficulty (Table 3.) and potentially compensated for deficient right dlPFC activity, the

analysis for effect of diagnosis suggests that the left occipital cortex may have served to support the visuo-spatial needs of the task.

2.5. Conclusions

First-episode schizophrenia subjects with and without a history of cannabis use and non-psychotic cannabis users failed to activate the dlPFC during processing of the most difficult TOL problems. As there were no significant differences between groups on behavioural performance of the task, the altered pattern of cortical activation to the TOL task suggests that neuroadaptation takes place with long-term cannabis use [24]. In schizophrenia right dlPFC activation was the main effect of diagnosis, suggesting an altered working memory load-response curve and a reliance on the posterior neural networks to successfully complete tasks of higher degrees of difficulty. The main effect of negative BOLD activation for both cannabis use and schizophrenia was in the frontal eye field area (BA 6 and 8), which may indicate an attenuation of activity of the visual attention and motor systems to enable increased capacity for task relevant processing. Therefore, alternative cortical recruitment strategies for solving visuo-spatial working memory problems may be available to schizophrenia patients and cannabis users alike. A trend towards more marked decrements in network activation for the cannabis using schizophrenia patients was also seen. These data may be consistent with the clinical literature that has reported poorer outcomes for patients with schizophrenia who also use cannabis and suggest the possibility of a detrimental synergistic effect on frontal lobe function. However, some of our findings need to be interpreted with caution. Since all schizophrenia patients in the current study were on maintenance antipsychotic drug treatment at the time of testing and as anti-dopaminergic drug actions interfere with information processing within the frontostriatal network [209], some of the group differences could reflect medication effects. Ideally, this study would have been conducted with neuroleptic naïve schizophrenia patients.

Studying cannabis users is also fraught with complexities. Firstly, controling for the pharmacology of the different varieties of cannabis was not possible. Secondly, an objective confirmation of duration of abstinence from cannabis was not available. Urinary cannabinoid metabolite levels are known to fluctuate between positive and

negative results for days depending on the previous magnitude and frequency of use [210, 211]. Furthermore, ascertaining the time frame of acute, chronic and withdrawal effects requires an assessment of kinetic/dynamic interactions far more sophisticated than can be provided by a positive or negative urine test [212]. These factors may have resulted in some variability in 24-hr clearance and associated residual psychotropic effects. Finally, the study lacks some power, thus restricting further post hoc analyses of the proposed compensatory mechanisms (e.g., regression analysis of prefrontal and parietal BOLD responses with diagnosis as covariate).

3. Cerebellar grey matter deficits in first-episode schizophrenia mapped using cortical pattern matching[3]

3.1. Introduction

Cerebellar dysfunction in schizophrenia has gained substantial attention in recent years (e.g., [213, 214], for a review) and led to the notion of "cognitive dysmetria"– a syndrome characterised by neurological soft signs, impaired coordination, abnormal posture and proprioception, impaired eye-blink conditioning, vestibule-ocular dysadaptation, and poor performance on procedural learning tasks. Post mortem micro- and macroscopic investigations identified the vermis region as most affected in schizophrenia. In their early studies, Weinberger and colleagues (1980) reported anterior vermal atrophy accompanied by Purkinje and granule cell loss, thus resulting in thinning of the granular and molecular layers ([215] for review). These early post mortem findings are consistent with reports derived from in vivo brain imaging research confirming vermal atrophy by computerised tomography (e.g., [216-219]) and magnetic resonance imaging (MRI; e.g., [220-228]). More recent reports also suggest an altered proportional relationship of vermal grey to white matter in schizophrenia (e.g., [229]).

Meta-analysis of voxel-based morphometry data by Honea and colleagues (2005) conducted on 15 studies with a total of 390 patients and 364 healthy volunteers confirmed predominantly left-hemispheric cerebellar atrophy in schizophrenia. However, two studies did not include the cerebellum and only four studies independently reported significant cerebellar atrophy. Posterior cerebellar atrophy and increased grey matter in the medial cerebellum and culmen was detected in a sample of 169 patients from the National Institute of Mental Health Genetic Study of

[3] Rasser, P.E, Schall, U., Peck, G., Cohen, M., Johnston, P., Khoo, K., Carr, V.J., Ward, P.B., Thompson, P.M. Cerebellar grey matter deficits in first-episode schizophrenia mapped using cortical pattern matching. *Neuroimage,* **2010**, 53:1175-1180

Schizophrenia when comparing patient MRI data with data from 212 healthy volunteers by optimised voxel-based morphometry [230]. Left cerebellar atrophy was also reported for 213 unaffected siblings, although this finding was not confirmed by intra-class correlation analysis conducted on 116 sibling pairs.

MRI studies have also investigated structural/functional relationships and, for instance, have shown that cerebellar atrophy is linked to impaired motor sequencing [231]. Other research has linked cerebellar dysfunction to neurological soft signs, abnormal posture and gait in schizophrenia ([213], for review). Moreover, functional brain imaging research has also provided some evidence for a cerebellar contribution to neurocognitive impairment in schizophrenia ([214], for review).

Clinical lesion studies [232] suggest that abnormal vermal-fastigial function can contribute to delusion symptoms, impaired control of attention and affect, and social dysfunction (including autistic spectrum symptoms and impaired Theory of Mind performance) via interactions with the anterior thalamic nuclei, hippocampus, septum, amygdala, ventral tegmental area, periaqueductal grey and mamillary bodies relevant for memory and emotion. This spectrum of putatively cerebellar psychopathology is largely consistent with some of the defining symptoms of schizophrenia, such as psychosis, inattention, flat affect, and social withdrawal.

When investigating associations of neurocognitive function with cerebral structure, Segarra and colleagues (2008) reported a correlation of vermal grey matter atrophy with working memory deficits [233]. By contrast, increased vermal white matter volumes have been linked to poor verbal fluency performance in schizophrenia [234]. Smaller volumes of the posterior and superior vermis were also found to correlate with impaired cognition in drug-naïve first-episode schizophrenia patients [228] while positive symptom ratings were reported to correlate with vermal white matter volumes in early-onset schizophrenia [235]. Moreover, cerebellar atrophy has also been detected in individuals followed longitudinally from the prodromal phase of the illness [236, 237]. However, in contrast to general cerebral atrophy, progression of vermal atrophy has not been confirmed by repeated MRI in

childhood-onset schizophrenia patients [237]. Together, these findings support the notion of clinically relevant cerebellar neuropathology in schizophrenia that is already present in the emerging phase of illness and, to some extent, also appears to be present in unaffected biological relatives [230].

The current study investigated regional cerebellar grey matter in well-remitted first-episode schizophrenia outpatients with less than 2 years' duration of illness by applying a novel MRI brain imaging analysing technique. This method has been widely used for mapping abnormalities of the cerebral cortex [238] and is applied here to the cerebellum for the first time.

The analysis methods preserve the three-dimensional lobular information of the cerebellum when generating statistical maps for group comparisons. In other words, data from the same lobules are averaged together across subjects when generating the average maps. Surface-based anatomical landmarks are used to enforce more accurate co-registration of individual lobule anatomy and more precise mapping of group-averaged surface anatomy. This is of particular importance when investigating potential regional grey matter differences in clinical populations where brain pathology can render more common co-registration algorithms less accurate, and where anatomical variance may be greater than in controls [239, 240].

It was hypothesised that there would be cerebellar grey matter deficits in first-episode schizophrenia that would also affect grey to white matter ratios [229], with the most pronounced regional grey matter deficit in the vermis area in this early phase of illness ([228]).

3.2. Methods

Ethics approval for this study was granted by the human research ethics committees of the University of Newcastle and Hunter New England Health. Participants gave written informed consent.

3.2.1. Subjects

Thirteen first-episode schizophrenia outpatients with less than two years' duration of illness since meeting DSM-IV criteria (Structured Clinical Interview for DSM-IV Axis I Disorders; [241]) participated in the study. Patients had to be 16 to 28 years old, in symptom remission following no more than one hospital admission and on maintenance atypical antipsychotic monotherapy (i.e. risperidone, olanzapine, aripiprazole, or quetiapine) at the time of recruitment into the study.

Patients were pair-wise matched to 13 healthy control subjects by age (mean: 21.6+/-S.D. 2.8 years; patients: 20.7+/-S.D. 3.6 years) and sex (12 males and 1 female) and are – with the exception of one subject – a subset of the 18 control subjects (mean age: 21.7+/-S.D. 2.3, 15 males and 3 females) who formed the standard average cerebellar reference atlas for this study. All subjects were right-handed (Edinburgh Handedness Scale; [185]).

Exclusion criteria for all participants included substance abuse or addiction (DSM-IV criteria), a history of significant head injury, relevant neurological (e.g., epilepsy) or medical (e.g., endocrine) conditions, ferromagnetic implants, claustrophobia or other anxiety disorders, a failure to complete at least three years of secondary school, or National Adult Reading Test (NART; [242]) IQ estimates of less than 70.

3.2.2. Magnetic resonance data acquisition and processing

MRI data were acquired using a Siemens Magnetom Vision 1.5 T MRI scanner (John Hunter Hospital, Newcastle). For each subject, high-resolution structural MR data was collected with approximate dimensions of 164 x 256 x 256 with each voxel being 1 x 1 x 1 mm using a 3D MPRAGE protocol with TR=9.7 msec, TE=4 msec, and flip angle=12 degrees. Subject's MRIs were transformed to ICBM [243] space using the software program register available at http://www.bic.mni.mcgill.ca/software/register/register.html. Following radio frequency bias correction [244], the cerebellum was manually traced blind to

diagnosis and isolated to extract a model of the cerebellar cortex [245] (Figure 5a & b). This model was used together with the subject's MRI to identify and trace the intra-culminate, primary, superior posterior, horizontal and secondary fissures (Figure 6a). A cortical pattern matching technique [179, 238] was then employed to create a geometric average target set of fissures to which each subject's cerebellar cortex model was deformed.

Figure 5: (a) Masked cerebellum in an MRI coronal section. (b) Extracted cerebellum coronal section (surrounding tissue removed). (c) Grey and white matter tissue classification by thresholding procedure [246]. Red circle illustrates spherical kernel of 15 mm radius (adjusted by individual total cerebellar volume) to calculate the proportion of grey matter within the kernel sphere for each cerebellar surface vertex.

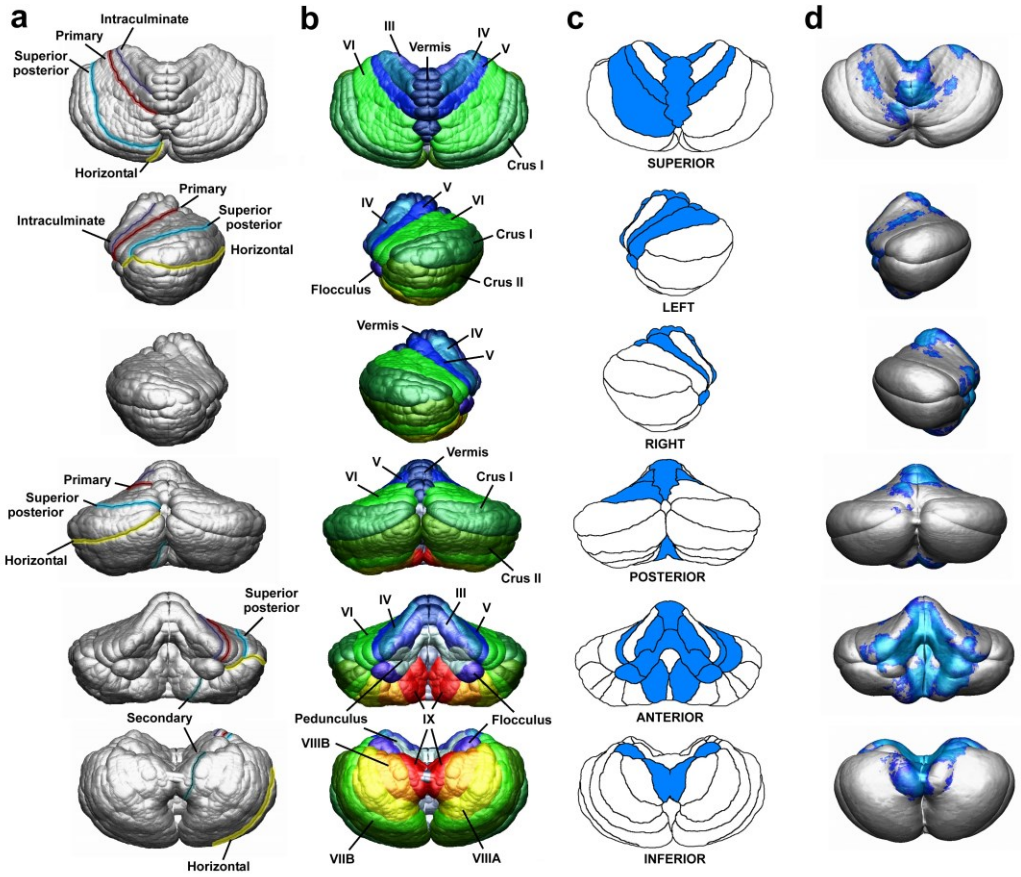


Figure 6: From top to bottom, superior, left, right, posterior, anterior, and inferior views of cerebellum. (a) Traced fissure lines (intra-culminate, primary, superior posterior, horizontal, and secondary) for cortex model transformation. (b) Deformable lobule labels, enabling lobule-wise grey matter measures for each subject. (c) Cerebellar lobules with significant grey matter reduction (blue) in first-episode schizophrenia. Left hemisphere: vermis, lobuli III, V, VI, IX, pedunculus, and flocculus. Right hemisphere: vermis, lobuli III, V, and IX, pedunculus, and flocculus. (d) Statistical maps of regional cerebellar grey matter deficits (P<0.05) in the superior vermis, the left lobuli VI, in right-inferior lobule IX, extending into left lobule IX, bilaterally in the areas of lobuli III, peduncle and in left flocculus.

In native space, a secondary correction of radio frequency bias was applied on the subject's MRI that included using a dilated version of their native space cerebellar mask. This was done to improve the homogeneity of the intensities within each tissue class across the cerebellum. The cerebellum was then isolated using the cerebellar mask, followed by intensity normalisation, and calculation of the total cerebellar volume for each subject.

Grey and white matter tissue was classified using a thresholding procedure [246] that employed a single Gaussian model for each subject (Figure 5c). This threshold, standardised across all subjects, was based on a constant offset from the mean of each subject's fitted Gaussian curve.

In native space, the proportion of cerebellar voxels labelled as grey matter was determined within volume-dependent spherical kernels with centres that correspond to the vertices of the subject's deformed cerebellar cortex model (Figure 5c). This is a standard approach in voxel-based morphometry studies and allows the regional assessment of gray matter volumes. The size of the spherical kernel for each subject was varied dependent on their total cerebellar volume, with the volume of the sphere kernel of radius 15 mm adjusted by the ratio of the individual to the average cerebellar volume determined from the reference sample of 18 control subjects.

A deformable cerebellar atlas was applied to label the cerebellar lobules of the average model, enabling lobule-wise averaging of the grey matter measure for each subject (Figure 6b).

The deformable atlas was generated from a three-dimensional model of the cerebellar cortex extracted [245] from a symmetrical version of the Montreal Neurological Institute intensity-averaged single-subject MRI [247] The symmetry was deliberately ensured by mirroring the right-hand side across the central sagittal plane.

The cerebellar fissures, as described in [248] were used to describe the lobule and feature boundaries on the cerebellar cortical surface model. The cerebellar model was then deformed using cortical pattern matching to the average target atlas followed by tabulation of each subject's average proportion of grey matter for each lobule.

3.2.3. Statistical analyses

Total cerebellar volume, total grey and white matter volumes and total grey to white matter ratios were *t*-tested at $P<0.05$ (two-tailed). Parametric statistical maps of

cerebellar grey matter measures by group were calculated and permutation-tested [249] at $P<0.05$ for each hemisphere. Permutation testing ascribes an overall corrected p-value to a whole map of statistics, based on estimating the chance that the overall surface area of suprathreshold statistics could have been obtained by chance in null data, simulated by randomly assigning patients and controls to two groups. Lobule-level group differences of grey matter were *t*-tested at $P<0.05$ (two-sided) and *Bonferroni*-corrected for multiple tests for each hemisphere.

3.3. Results

Total cerebellar volume and total grey matter volume of first-episode schizophrenia patients did not differ from healthy control subjects (Table 5). By contrast, first-episode schizophrenia was associated with increased total white matter volume ($t=-2.17$; $P<0.05$) and smaller total grey to white matter ratios ($t=2.91$; $P<0.01$).

Grey matter deficits in first-episode schizophrenia patients were confirmed by permutation testing at $P<0.005$ for the left and at $P<0.003$ for the right hemisphere of the cerebellum (Figure 6d). Four clusters of cerebellar grey matter reduction were identified: (i) in superior vermis; (ii) in the left lobuli VI; (iii) in right-inferior lobule IX, extending into left lobule IX; (iv) bilaterally in the areas of lobuli III, peduncle and left flocculus (Figure 6d).

This topographic pattern of grey matter reduction in first-episode schizophrenia was also confirmed at $P<0.05$ at the lobule level (Figure 6c). In the left cerebellar hemisphere, reduced grey matter was present in vermis, lobuli III, V, VI, IX, pedunculus, and flocculus (Table 6). Right hemispheric grey matter reductions were found in vermis, lobuli III, V, and IX, pedunculus, and flocculus (Table 7). When applying *Bonferroni* correction for multiple comparisons, grey matter reductions in patients were confirmed at $P<0.004$ for right lobuli III and IX, left flocculus and pedunculi in both hemispheres.

Table 5: Comparisons of total cerebellar volumes, total grey and white matter volumes, and grey to total cerebellar volume ratios (standard deviations are shown in parentheses).

	Control Subjects (*N*=13)	First-episode Schizophrenia Patients (*N*=13)	*t*-statistic (*df*=24)	*P* *(2-sided)*
Total Cerebellar Volume (cc)	145.9 (11.7)	149.5 (10.5)	-0.82	0.42
Total Grey Matter Volume (cc)	104.9 (8.5)	105.0 (7.3)	-0.04	0.96
Total White Matter Volume (cc)	41.0 (3.7)	44.4 (4.3)	-2.17	0.04
Total Grey to Total Cerebellar Volume Ratio	0.72 (0.01)	0.70 (0.02)	2.91	0.008

Table 6: Comparison of grey matter proportion by lobule (standard deviation) in the left cerebellar hemisphere

Lobules (Left Hemisphere)	Control Subjects (*N*=13)	First-episode Schizophrenia Patients (*N*=13)	*t*-statistic (*df*=24)	*P* *(2-sided)*
III	0.73 (0.03)	0.69 (0.04)	3.09	0.005
IV	0.78 (0.04)	0.75 (0.04)	1.60	0.12
V	0.75 (0.03)	0.72 (0.03)	2.18	0.04
VI	0.75 (0.02)	0.73 (0.02)	2.50	0.02
Pedunculus	0.65 (0.03)	0.60 (0.02)	4.78	<0.001*
Crus I	0.79 (0.02)	0.78 (0.03)	0.86	0.40
Crus II	0.76 (0.02)	0.76 (0.02)	0.63	0.54
VIIB	0.76 (0.02)	0.75 (0.03)	0.33	0.74
VIIIA	0.76 (0.02)	0.75 (0.04)	0.75	0.46
VIIIB	0.77 (0.02)	0.75 (0.04)	1.16	0.11
IX	0.78 (0.01)	0.75 (0.03)	3.10	0.005
Vermis	0.80 (0.03)	0.76 (0.03)	3.10	0.005
Flocculus	0.78 (0.01)	0.75 (0.02)	4.13	<0.001*

*Significant following *Bonferroni correction* (*P*<0.004)

Table 7: Comparison of grey matter proportion by lobule (standard deviations are in parentheses) in the right cerebellar hemisphere.

Lobules (Right Hemisphere)	Control Subjects (*N*=13)	First-episode Schizophrenia Patients (*N*=13)	*t*-statistic (*df*=24)	*P* *(2-sided)*
III	0.74 (0.03)	0.69 (0.03)	3.82	0.001*
IV	0.78 (0.03)	0.76 (0.03)	1.88	0.07
V	0.75 (0.03)	0.72 (0.02)	2.40	0.02
VI	0.75 (0.03)	0.73 (0.02)	1.81	0.08
Pedunculus	0.65 (0.03)	0.61 (0.02)	4.77	<0.001*
Crus I	0.79 (0.02)	0.79 (0.03)	0.06	0.95
Crus II	0.77 (0.02)	0.77 (0.02)	0.11	0.91
VIIB	0.76 (0.02)	0.75 (0.03)	0.70	0.49
VIIIA	0.77 (0.03)	0.76 (0.04)	1.07	0.29
VIIIB	0.78 (0.03)	0.76 (0.03)	1.91	0.07
IX	0.81 (0.02)	0.78 (0.03)	3.19	0.004*
Vermis	0.80 (0.03)	0.77 (0.03)	2.97	0.007
Flocculus	0.64 (0.03)	0.61 (0.02)	3.12	0.005

*Significant following *Bonferroni correction* (*P*<0.004)

3.4. Discussion

In terms of overall volume, the cerebellum was not atrophic in first-episode schizophrenia, but patients exhibited increased total white matter volumes, resulting in smaller global grey to white matter ratios. Regional grey matter deficiencies were also detected. These findings suggest that grey matter loss contributing eventually to global cerebellar atrophy may be partly compensated initially by increased volumes of global white matter and, furthermore, that grey matter deficits are regionally circumscribed to areas of the cerebellum linked to symptoms and cognitive deficits as they are expressed in established schizophrenia. Our findings further demonstrate that cerebellar pathology has already emerged in the early first-episode stage of the condition.

Consistent with previous findings [228], our data confirm cerebellar grey matter reduction in the superior vermal region. The vermis is mainly involved in spinocerebellar processing of axial muscle coordination. According to Schmahmann et al. (2008) neuropathology in vermis has also been linked to impaired attention control, dysregulation of affect, social dysfunction and delusions. This spectrum of psychopathology is consistent with some of the positive (e.g., delusions and inattention) and negative symptoms (e.g., flat affect and social withdrawal) of schizophrenia. Other reports found correlations of reduced vermal volume with the depression and paranoia sub scores of the Brief Psychiatric Rating Scale [223] and greater vermis white matter volumes with severity of positive symptoms, thought disorder and impaired verbal logical memory in patients with schizophrenia [250].

However, our study lacks the power to detect any potential association of symptom expression with morphological data, particularly since our first-episode cohort displayed very little ongoing psychopathology when it was investigated following recovery from their first psychotic episode. Notwithstanding, the areas of regional cerebellar grey matter deficits in this early phase of confirmed schizophrenia are nevertheless consistent with functional deficits as they have been described for established and more chronic forms of illness.

For instance, our data indicate pronounced grey matter deficits in the flocculus bilaterally. The flocculus and paraflocculus, together with anterior vermis, are involved in the execution of smooth pursuit eye movements, preventing blurring of the retinal image of moving objects. This process integrates retinal image motion signals with ongoing eye and head movements in frontal and supplementary eye fields, medial superior temporal and ventral intraparietal cortex. The flocculus and paraflocculus receive cortical input via the pontine nuclei for processing visual and vestibulo-ocular information and to the vermis for pursuit initiation [251].

Impaired smooth pursuit eye movements are well documented in schizophrenia (see [252], for review), including in first-episode patients (e.g., [253, 254]). Reduced grey matter in the flocculus region suggests local cerebellar neuropathology consistent with a pursuit deficit.

Moreover, our data also suggest grey matter deficits in this early phase of illness in cerebellar pedunculi. Functionally, the pedunculi are involved in associative learning, such as the formation of memory traces for the conditioned eye-blink response [240]. A previous report described lower rates of eye-blink conditioning, particularly less adaptively timed conditioned response latencies [255] in schizophrenia. The current finding of reduced grey matter in pedunculi further suggests cerebellar pathology that is already emerging in this area following the first episode of illness.

However, the current data suggest even more widespread cerebellar neuropathology in this early phase of schizophrenia affecting lobuli III, V, VI, and IX. Some of these cerebellar regions are involved in somatosensory, language, verbal working memory, spatial, and executive cognitive functions and the processing of emotional information [256]. When reviewing functional brain imaging data however, function/structure associations are less well-defined and depend on task type and cognitive domain. Most studies have not used methods capable of precisely mapping subtle regional morphological differences. Cortical pattern averaging overcomes some of these limitations, allowing a more accurate mapping of grey matter abnormalities in relatively small patient cohorts. This suggests the promise

of investigating function/structure associations in more detail. Such research should also include corresponding morphological measures from the cerebrum in order to investigate the contribution of individual cortico-cerebellar circuits to functional deficits and clinical features of the disorder.

Another inherent problem with studies on schizophrenia patients is their treatment history. While vermal volume reduction has been found in neuroleptic-naïve schizophrenia patients [223, 228], antipsychotic medication is a potential confound associated with cerebral grey matter reduction [257]. This finding must be considered when interpreting structural brain imaging data in schizophrenia patients. Even so, the patients in this study were treated for a relatively short period of time, using atypical antipsychotics that are thought to have less pronounced effects on grey matter changes [258].

In summary, cortical pattern matching overcomes some limitations of other morphometric methods by accurately aligning group-averaged cortical MRI data in relatively small cohorts. The findings confirm cerebellar grey and white matter pathology in schizophrenia [213, 214]; these deficits are already detectable in the first-episode phase of the illness, following remission from acute psychosis. These findings are consistent with the notion that the cerebellum contributes to the clinical, cognitive, and pathophysiological signs of the disorder. However, further research is required to confirm the association of functional deficits with morphological differences in the cerebrum and cerebellum. Future studies should also target potentially confounding medication effects as well as longitudinal morphological changes with progression of illness.

4. Cerebellar grey matter deficits, cannabis use and first-episode schizophrenia in adolescents and young adults[4]

4.1. Introduction

As already highlighted in Chapter 1 and 2, cannabis is the most commonly used illicit drug, abused by approximately 4% of the adult population worldwide [259]. Particularly high rates are recorded for North America and Oceania. Approximately 40% of the North American population aged 12 years and older [260] and 34% of the Australian population aged 14 years and older [261] had a reported lifetime use of cannabis. The European Union recorded lower rates at 22% for the age group 15-64 years [262]. Of particular concern is the increasing trend for using hydroponically grown cannabis with significantly higher tetrahydrocannabinol (THC) content at younger ages (see review [263]).

Meta-analyses by Moore and colleagues [145] show that there is an increased risk of psychosis (i.e., beyond the immediate impact of intoxication) in individuals who had ever used cannabis and a greater risk in people who have used cannabis more frequently. Although the authors concluded that an association with mood and affective disorders was less clear, the observation of increased risk of psychosis raises health concerns for the mental wellbeing of young regular users of cannabis.

Cannabis is a plant derivative of Cannabis sativa, indica, and ruderalis which differ in their respective THC and cannabidiol content. THC is the main psychoactive compound and acts on the CB1 cannabinoid receptor in the nervous system. In the human brain, the highest density of CB1 receptors is found in the midbrain (i.e., substantia nigra, red nucleus, central grey, and superior colliculus), followed by the

[4] Cohen, M., Rasser, P.E., Peck, G., Carr, V.J., Ward, P.B., Thompson, P.M., Johnston, P.J., Baker, A., Schall, U. Cerebellar grey matter deficits, cannabis use and first-episode schizophrenia in adolescents and young adults. *International Journal of Neuropsychopharmacology,* **2012**, 15:297-307

basal ganglia (i.e., globus pallidus) and the molecular layer of the cerebellum. Lower densities are found in Layer I of the primary and secondary visual cortex [264].

In the adult brain, endocannabinoids (e.g., anandamide and 2-arachnidonoylglycerol) mediate retrograde synaptic signalling, thereby controlling synaptic plasticity. CB1 receptors play an integral role in synapto-, neuro- and morphogenesis as well as neuronal migration and specification during brain development [265]. Postnatal brain maturation is largely defined by myelination and synaptic pruning. These processes result in grey matter thinning and a white matter volume increase (e.g. [266]).

Of particular interest are the neuro-developmental processes of adolescence and early adulthood when schizophrenia emerges and cannabis abuse is most prevalent. The role of the endocannabinoid system in this phase of brain maturation remains unclear, but the continued expression of CB1 receptors in brain regions that change during adolescence suggests an ongoing role, e.g., in synapse formation [265].

There is clear evidence for substantial structural brain changes in first-episode schizophrenia (FES), including some evidence suggesting that these changes already emerge in the prodromal phase of illness (e.g., [267-270]). Rais and colleagues further reported that first-episode schizophrenia patients who have used cannabis show a more pronounced brain volume reduction over a 5-year period than patients with schizophrenia who did not use cannabis [271]. Whilst these authors did not report cerebellum data, cerebellar atrophy has been detected in individuals followed longitudinally from the prodromal phase of the illness [236, 272]. Progression of vermal atrophy in childhood-onset schizophrenia patients has not been confirmed by repeated MRI [237]. Together, these findings support the notion of cerebellar neuropathology in schizophrenia that is already present in the emerging phase of illness. We investigated whether a history of juvenile cannabis consumption in the prodromal phase of illness was also associated with more pronounced cerebellar neuropathology in FES as reported for the cerebrum [271].

Cannabis-induced brain changes in otherwise healthy individuals are comparatively less prominent (for review see [273]). Reported cannabis-induced brain changes range from altered gyrification in young users, consistent with neurodevelopmental abnormalities [274], to volumetric reductions of amygdala and hippocampus in an exposure-dependent fashion [29]. In the latter study, sub-threshold positive symptoms were also correlated with left-hemispheric hippocampal volume reduction and cumulative lifetime exposure to cannabis. Compared to studies of structural brain changes, functional brain imaging studies are more common. These predominantly show reduced prefrontal blood flow in cannabis users compared to non-users (for review see [273]). However, there are very few studies specifically investigating effects of juvenile cannabis use on the cerebellum. For instance, [275] reported larger vermal volumes in recently abstinent cannabis users, along with impaired executive functions, which is in contrast to the findings reported for the cerebrum showing smaller regional volumes with cannabis use in a dose-dependent fashion (e.g. [29]).

The current study investigates the neurodevelopmental effects of juvenile cannabis use on the CB1-rich grey matter of the cerebellum [264] in a group of young people with heavy cannabis abuse during adolescence. It was hypothesised that cerebellar grey matter reduction would be related to: (1) age at onset of cannabis use (i.e., greater reduction when use starts at a younger age); (2) duration of cannabis use (i.e., more grey matter reduction with longer use); and (3) the total amount of accumulated lifetime dose (i.e., dose-dependent reduction of grey matter).

Also examined was the effect of cannabis use on cerebellar pathology in young people diagnosed with FES (e.g., [228, 276, 277]). It was hypothesised that (4) FES cannabis users would show greater regional grey matter reduction in the cerebellum when compared to a group of non-using FES, and that (5) cannabis use contributes to and overlaps with the cerebellar grey matter reduction pattern found in non-using FES. The latter hypothesis is reflecting the notion of a promoting effect of juvenile cannabis use on cerebellar pathology as, for instance, [278] reported as decreased grey matter density in the CB1 receptor-rich region of right posterior

cingulate cortex when employing a voxel-based morphometric analysis and comparing cannabis-using with non-using FES patients.

4.2. Methods

Ethics approval for this study was granted by the human research ethics committees of the Universities of Newcastle and New South Wales, Hunter New England Health, and South Eastern Sydney Area Health. Participants gave written informed consent.

4.2.1. Subjects

Nineteen healthy non-drug using volunteers (including 13 participating in the previous study), 17 young cannabis users, 13 non-using and 6 cannabis-using FES outpatients were recruited through community advertisement. FES patients participated in the previous study. They had a history of less than five years since their first psychotic episode and less than two years since meeting DSM-IV criteria for schizophrenia [241]. All four participant groups were closely matched for age, gender and handedness as well as for cannabis-use history (for the cannabis using subjects) and duration of illness (for the FES subjects; Table 8). FES participants were in remission after no more than one hospital admission and were maintained on atypical antipsychotic monotherapy (i.e., risperidone, olanzapine, aripiprazole, or quetiapine) when recruited into the study.

The Opiate Treatment Index [279] was used to assess illicit and licensed drug use and to estimate the quantity/frequency of cannabis use expressed as individual consumption events and referred to as “doses” ([279], Table 8). All participants were right-handed (Edinburgh Handedness Scale; [185]). Exclusion criteria for all participants included non-cannabis forms of substance abuse or dependence, other than caffeine or nicotine according to DSM-IV criteria. As a result, the groups did not differ on any substance use patterns other than cannabis (Table 8). Other exclusion criteria included: a history of significant head injury, relevant neurological (e.g., epilepsy) or medical (e.g., endocrine) conditions, ferromagnetic implants,

claustrophobia or other anxiety disorders, failure to complete at least three years of secondary school, or National Adult Reading Test [242] IQ estimates of less than 70.

Table 8: Demographic, handedness, age at psychosis onset, cannabis, alcohol and tobacco use data (standard deviations are in parentheses; SD) for healthy control subjects, cannabis using subjects, first-episode schizophrenia (FES) subjects, and cannabis using FES subjects.

	Control Subjects	Cannabis Using Subjects	FES	Cannabis Using FES	*P*
Male/Female	15/4	15/2	12/1	4/2	0.64[#]
Age (SD)	21.5 (2.3)	22.7 (2.4)	20.7 (3.6)	21.8 (1.9)	0.24[$]
Handedness (R/L)	19/0	17/0	13/0	6/0	-
Age at First Psychotic Episode (SD)	-	-	17.5 (2.5)	17.4 (2.2)	0.96[$]
Age at Onset of THC USE (SD)	-	15.1 (2.4)	-	15.5 (2.3)	0.74[$]
Years of THC Use (SD)	-	7.6 (2.6)	-	6.3 (2.3)	0.30[$]
Estimated Total Lifetime THC Doses (SD)	-	22,700 (16,400)	-	17,900 (19,000)	0.56[$]
Estimated Alcohol Consumption in grams/day (SD)	11.2 (14.5)	24.1 (24.0)	18.0 (6.0)	29.7 (27.2)	0.09[$]
Daily Tobacco Consumption (Y/N)	7/12	13/4	5/8	3/6	0.14[#]

[#]Chi^2 Test (*Yates*-corrected); [$]One-way ANOVA

4.2.2. Magnetic resonance data acquisition and processing

The methods closely follow those described in the previous study. MRI data were acquired using a Siemens Magnetom Vision 1.5 T MRI scanner (John Hunter Hospital, Newcastle). High-resolution structural brain MR data were collected from each subject with approximate dimensions of 164 x 256 x 256 with each voxel being 1 x 1 x 1 mm. We used a 3D MPRAGE (magnetization prepared gradient echo) protocol with repetition time (TR)=9.7 msec, echo time (TE)=4 msec, and flip angle=12 degrees.

Pre-processing of the subject's MRI scan followed a protocol [280] involving transformation to ICBM space [243], radio frequency bias correction [244], manual delineation of the cerebellum (blind to diagnosis) followed by the extraction of a surface-based model of the cerebellar cortex [245]. Using the surface model and all 3 triaxial sections of the subject's MRI, five cerebellar fissures (the intra-culminate, primary, superior posterior, horizontal and secondary) were identified and traced onto their cerebellar model (Figure 7 A). A cortical pattern matching technique [238, 281] was then employed to deform each subject's cerebellar model to a geometrically averaged target set of fissures.

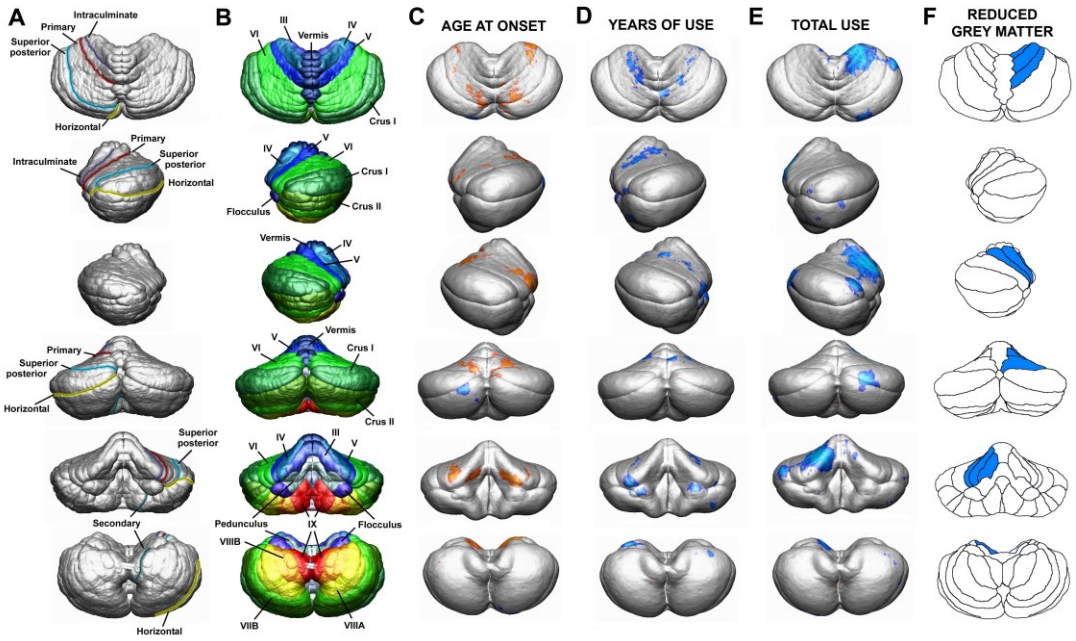


Figure 7: From top to bottom, superior, left, right, posterior, anterior, and inferior views of cerebellum. (A) Traced fissure lines (intra-culminate, primary, superior posterior, horizontal, and secondary) for cortex model transformation (Modified from [281] (B) Deformable lobule labels, enabling lobule-wise grey matter measures for each subject (Modified from [281]). Correlation maps of cerebellar grey matter with (C) age of onset of cannabis (THC) use, (D) years of use, and (E) cumulated life-time exposure. (F) Cannabis use history effects of juvenile THC use were statistically confirmed for the right lobules III, IV, and V.

Using a dilated version of their native space cerebellar mask, a secondary correction for radio frequency bias (intensity inhomogeneity) was applied to the subject's MRI to improve the homogeneity of intensities within each tissue class. Each subject's cerebellum was then isolated using their cerebellar mask, followed by intensity normalisation, and total cerebellar volume was calculated.

Cerebellar grey and white matter tissues were classified using a thresholding procedure [246]. This procedure divided the tissue classes based on a constant offset from the mean of a single fitted Gaussian model for each subject. Tissue-classified volumes were used to measure total cerebellar grey and white matter volumes.

For the regional assessment of grey matter in native space, the proportion of cerebellar voxels labelled as grey matter was calculated within volume-dependent spherical kernels with centres corresponding to the vertices of the subject's deformed cerebellar cortex model. These spherical kernels, with radius 15 mm, were varied subject-wise by the ratio of the individual to the average cerebellar volume, with the average determined from a reference sample [281]of 18 control subjects also belonging to this study.

A deformable cerebellar atlas (Figure7 B) [281] was then deformed using cortical pattern matching to the average target atlas, enabling the tabulation of each subject's average proportion of grey matter for each lobule. The deformable atlas was generated from a symmetrical version (across the central sagittal plane) of the Montreal Neurological Institute intensity-averaged single-subject MRI [247]. Labelling of lobule and feature boundaries of the deformable atlas was as described in Schmahmann et al., [248].

4.2.3. Statistical analyses

Demographic data were compared between groups by non-parametric testing (i.e., Chi^2 statistics with *Yates* correction when indicated) for nominal data or one-way ANOVA at $p<0.05$ (two-sided). Data distribution was tested prior to parametric testing. Associations between parameters describing cannabis use history (i.e. age of onset and duration of cannabis use and estimated individual total lifetime doses) were tested with *Pearson* correlation coefficients at $p<0.05$ (two-sided) in the cannabis-using group. Total cerebellar volume, total grey and white matter volumes, ratios of total grey to total cerebellar volumes, and lobule-level group differences of grey matter were tested by one-way ANOVA at $p<0.05$ (two-sided and *Bonferroni*-corrected for multiple comparisons) between all four groups (i.e. non-using healthy control subjects, cannabis using subjects, first-episode schizophrenia subjects, and cannabis-using first-episode schizophrenia subjects). This was followed by *Scheffe* post hoc group comparisons and linear regression with the parameters describing cannabis use history as predictors and lobule-level grey matter differences as the

dependent variable. The respective main effects and interaction between diagnosis of first-episode schizophrenia and cannabis use was tested by two-way ANOVA at $p<0.05$ (two-sided) across all four groups.

Parametric statistical maps of cerebellar grey matter measures by group (i.e. non-using healthy control subjects, cannabis using subjects, first-episode schizophrenia subjects, and cannabis-using first-episode schizophrenia subjects) were calculated and permutation-tested [249] at $p<0.05$ for each hemisphere. Permutation testing ascribes an overall corrected p-value to a whole map of statistics, based on estimating the chance that the overall surface area of suprathreshold statistics could have been obtained by chance in null data, simulated by randomly assigning patients and controls to two groups.

4.3. Results

There was mixed support for the hypotheses. Cannabis use did not affect total cerebellar volume, white or grey matter volumes, nor total grey to total cerebellar volume ratios (Table 9). Parametric mapping suggested some regional effects of cannabis use on grey matter depending on age at onset of use, years of use, and accumulated total lifetime doses (Figure 7 C-E). Permutation testing confirmed significant grey matter reduction for total lifetime dose for the right cerebellar hemisphere ($p=0.04$; Figure 7 E).

Table 9: Comparisons of total cerebellar volumes, total grey and white matter volumes, and grey to total cerebellar volume ratios (standard deviations are in parentheses) for healthy control subjects, cannabis using subjects, first-episode schizophrenia (FES) subjects, and cannabis using FES subjects.

	Control Subjects (*N*=19)	Cannabis Using Subjects (*N*=17)	FES (*N*=13)	Cannabis Using FES *(N=6)*	*F* (*df*=3,54)	*P*
Total Cerebellar Volume (cc)	142.2 (13.1)	152.2 (13.9)	149.5 (10.5)	145.1 (7.4)	2.2	0.10
Total Grey Matter Volume (cc)	102.2 (10.0)	108.7 (9.7)	105.0 (7.3)	102.4 (5.4)	1.7	0.17
Total White Matter Volume (cc)	40.0 (3.7)	43.5 (4.5)	44.4 (4.3)	42.7 (2.4)	3.8	<0.02*
Total Grey To Total Cerebellar Volume Ratio	0.72 (0.01)	0.71 (0.01)	0.70 (0.02)	0.71 (0.01)	4.7	<0.01*

* *Scheffe* post hoc testing confirms increased total white matter volume (p=0.03) and decreased total grey to total cerebellar volume ratio (p=0.007) in FES without cannabis use versus control subjects

The parameters describing cannabis use were partially inter-correlated in the otherwise healthy cannabis using group. Age at onset of cannabis use correlated with years of use (r=-.52; p=0.03) which, in turn, correlated with total individual lifetime doses (r=.58; p=0.01). Hence, post hoc statistical analyses at lobule level were performed by linear regression with age of onset, years of use, and total

lifetime doses as predictors and grey matter measures in individual cerebellar lobules and areas as defined by Schmahmann et al., [248] as the dependent variable. Consistent with parametric mapping (Figure 7 C-E), grey matter reduction was confirmed for three lobules as dependent on the three parameters describing the pattern of cannabis use history (lobule III: $F(3,16)=4.4$, $p=0.02$; lobule IV: $F(3,16)=4.7$, $p=0.02$; lobule V: $F(3,16)=4.6$, $p=0.02$; Figure 7 F) with total lifetime doses significantly predicting grey matter reduction in these lobules (lobule III: $\beta=-0.63$, $t=-2.6$, $p=0.02$; lobule IV: $\beta=-0.74$, $t=-3.1$, $p<0.01$; lobule V: $\beta=-0.52$, $t=-2.2$, $p<0.05$; Supplementary Figure S1). Age at onset of cannabis use also showed a statistically non-significant trend towards being associated with grey matter reduction in lobule III ($\beta=-0.46$, $t=-2.0$, $p<0.07$; Supplementary Figure S2).

Parametric mapping suggested no regional differences in cerebellar grey matter in cannabis users versus pair-wise age, gender, and handedness-matched healthy control subjects (Figure 8 A). By contrast, FES subjects showed a marked reduction of grey matter in the superior vermis, in left lobule VI, in right-inferior lobule IX, extending into left lobule IX, bilaterally in the areas of lobuli III, peduncle and left flocculus, when compared to pair-wise age-, gender-, and handedness-matched healthy controls (Figure 8 B). These grey matter deficits were confirmed by permutation testing for the left ($p<0.003$) and right ($p<0.005$) cerebellar hemispheres.

Grey matter reductions in FES were also confirmed by parametric mapping across the three groups (i.e., healthy control, cannabis user, and FES subjects) by permutation testing for the left ($p<0.002$) and right ($p<0.003$) cerebellar hemispheres (Figure 2 C). Moreover, FES subjects also showed greater total white matter volume ($p=0.03$) and lower total grey to total cerebellar volume ratio ($p=0.007$) than healthy controls (Table 9).

To assess the respective contributions of cannabis use and schizophrenia to regional grey matter changes, all study participants were entered into a two-way ANOVA with the independent factors of cannabis use (yes or no) and the diagnosis of schizophrenia (yes or no). A main effect of the diagnosis of schizophrenia was

confirmed for total grey to total cerebellar volume ratios (F(1,51)=10.6, p=0.002; Supplementary Figure S3) due to grey matter loss on lobules III, IV, V, VI, IX, pedunculi, flocculi, and vermis (F(1,51)=4.5-14.3, p<0.05-<0.001; Figure 8 D – light blue areas) which corresponds to the main effect of group in lobules III, IX, pedunculi, flocculi, and vermis (F(3,51)=2.8-5.8, p<0.05-0.002; Figure 8 D – dark blue areas). After correcting for multiple testing *(Bonferroni)*, smaller grey matter was confirmed in FES subjects without cannabis use versus healthy control subjects, in the pedunculi, vermis and right lobule III (Table 10). There was neither an effect of cannabis use (F(1,51)=0.02) nor an interaction of cannabis use with the diagnosis of schizophrenia on regional grey matter differences in the cerebellum (F(1,51)=0.84).

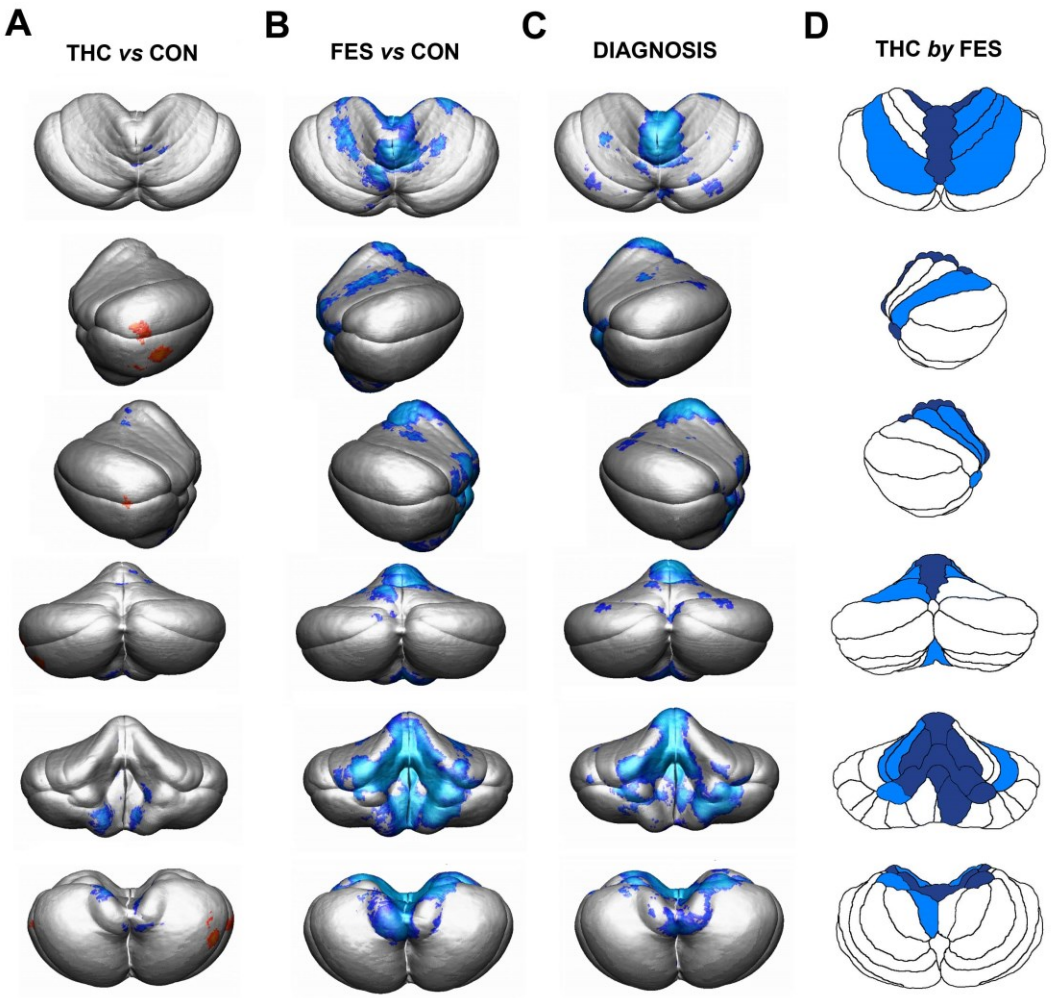


Figure 8: From top to bottom, superior, left, right, posterior, anterior, and inferior views of cerebellum. (A) Parametric mapping suggests no regional differences of cerebellar grey matter in cannabis users (THC) versus pair-wise age, gender, and handedness-matched healthy control subjects (CON). (B) By contrast, first-episode schizophrenia subjects (FES) present with marked reduction of grey matter in superior vermis; in left lobule VI, in right-inferior lobule IX, extending into left lobule IX, bilaterally in the areas of lobuli III, peduncle and left flocculus when compared to pair-wise age, gender, and handedness-matched healthy control subjects (CON) (Modified from [281]. (C) A main effect of the diagnosis of schizophrenia was confirmed (D) due to grey matter loss on lobules III, IV, V, VI, IX, pedunculi, flocculi, and vermis (light blue areas) which corresponds to the main effect of diagnosis of FES (2-way ANOVA of THC use by diagnosis of schizophrenia) in lobules III, IX, pedunculi, flocculi, and vermis (dark blue areas).

Table 10. Comparison of grey matter proportion by lobule (standard deviations are in parentheses) in the (A) left and (B) right cerebellar hemispheres for healthy control subjects, cannabis using subjects, first-episode schizophrenia (FES) subjects, and cannabis using FES subjects.

Lobules	Control Subjects (*N*=19)	Cannabis Using Subjects (*N*=17)	FES Subjects (*N*=13)	Cannabis Using FES Subjects *(N=6)*	*F* (*df*=3,54)	*P*
(A) Left Hemisphere						
III	0.72 (0.04)	0.71 (0.04)	0.69 (0.04)	0.71 (0.02)	2.8	<0.05
IV	0.78 (0.04)	0.78 (0.04)	0.75 (0.04)	0.77 (0.03)	1.6	0.19
V	0.75 (0.03)	0.74 (0.03)	0.72 (0.03)	0.74 (0.03)	2.2	0.10
VI	0.75 (0.02)	0.75 (0.03)	0.73 (0.02)	0.74 (0.03)	2.3	0.09
Pedunculus	0.64 (0.03)	0.64 (0.03)	0.60 (0.02)	0.63 (0.03)	5.4	<0.01*
Crus I	0.79 (0.02)	0.79 (0.02)	0.78 (0.03)	0.78 (0.02)	0.5	0.68

Crus II	0.76 (0.02)	0.77 (0.02)	0.76 (0.02)	0.76 (0.01)	0.3	0.82
VIIB	0.76 (0.02)	0.76 (0.02)	0.75 (0.03)	0.76 (0.01)	0.2	0.91
VIIIA	0.76 (0.02)	0.76 (0.03)	0.75 (0.04)	0.76 (0.01)	0.4	0.73
VIIIB	0.77 (0.02)	0.77 (0.03)	0.75 (0.04)	0.76 (0.02)	1.0	0.40
IX	0.78 (0.02)	0.76 (0.03)	0.76 (0.03)	0.76 (0.04)	3.0	0.04
Vermis	0.80 (0.03)	0.78 (0.03)	0.76 (0.03)	0.77 (0.03)	3.2	0.03*
Flocculus	0.66 (0.03)	0.66 (0.04)	0.63 (0.03)	0.63 (0.05)	3.3	0.03

(B) Right Hemisphere						
III	0.73 (0.04)	0.72 (0.04)	0.69 (0.03)	0.69 (0.03)	4.4	<0.01*
IV	0.78 (0.03)	0.78 (0.04)	0.76 (0.03)	0.74 (0.04)	2.2	0.10
V	0.75 (0.03)	0.75 (0.03)	0.72 (0.03)	0.74 (0.03)	2.6	0.05
VI	0.75 (0.03)	0.75 (0.03)	0.73 (0.02)	0.73 (0.02)	2.0	0.13
Pedunculus	0.65 (0.03)	0.64 (0.04)	0.61 (0.02)	0.61 (0.03)	5.8	<0.01*
Crus I	0.79 (0.02)	0.79 (0.02)	0.79 (0.03)	0.77 (0.00)	0.8	0.49

Crus II	0.77 (0.02)	0.77 (0.01)	0.77 (0.02)	0.76 (0.01)	0.7	0.54
VIIB	0.76 (0.03)	0.76 (0.02)	0.75 (0.03)	0.75 (0.02)	0.2	0.92
VIIIA	0.77 (0.03)	0.77 (0.02)	0.76 (0.04)	0.78 (0.02)	0.9	0.44
VIIIB	0.78 (0.02)	0.77 (0.02)	0.76 (0.03)	0.78 (0.03)	1.6	0.20
IX	0.80 (0.02)	0.79 (0.02)	0.78 (0.03)	0.79 (0.01)	2.8	0.50
Vermis	0.80 (0.03)	0.79 (0.03)	0.77 (0.03)	0.77 (0.03)	3.9	0.01*
Flocculus	0.64 (0.03)	0.64 (0.03)	0.62 (0.02)	0.62 (0.06)	2.8	0.05

* *Bonferroni*-corrected p-value. *Scheffe* post hoc testing confirms smaller grey to white matter ratios in FES without cannabis use versus healthy control subjects in left (p=0.008) and right (p=0.02) pedunculus, left (p=0.04) and right (p=0.03) vermis, and right lobule III (p=0.02)

4.4. Discussion

This is the first study to link cerebellar pathology to juvenile cannabis use by employing cortical pattern matching. Small dose-dependent regional effects of cannabis use in the cerebellum was found, resulting in reduced grey matter in the right lobules III, IV, and V that also tends to be more profound in lobule III with an earlier onset age of cannabis use. Despite dose-dependent regional grey matter reduction in cannabis users, overall grey matter measures were within the normal range of grey matter variability for healthy subjects. By contrast, Medina et al. (2010) reported larger inferior posterior vermis volumes in recently abstinent cannabis users when comparing to a closely matched non-cannabis using cohort. Their volumetric approach, however, does not discriminate between grey and white matter, nor does it adjust for individual lobular morphology when calculating group

averages, thereby limiting the comparability with the cortical pattern averaging method of the current study. On the other hand, remitted FES patients in the current study showed profound regional grey matter reduction in the vermis, pedunculi, and right lobule III at an early stage of illness. The cerebellum was not atrophic in FES, in terms of overall volume, but patients had greater total white matter volumes, resulting in smaller grey to total cerebellar volume ratios suggestive of impaired brain development [282].

The current findings confirm cerebellar grey and white matter pathology in schizophrenia [213, 214]. Moreover, these deficits are already detectable in the first-episode phase of the illness, following remission from acute psychosis. These findings are also consistent with findings from previous studies showing that the cerebellum contributes to the clinical, cognitive, and pathophysiological signs of the disorder. For instance, vermal neuropathology has been reported previously and also appears to be present in neuroleptic-naïve schizophrenia patients [223, 228]. Functionally, the vermis is mainly involved in spinocerebellar processing of axial muscle coordination but has also been linked to impaired attention control, dysregulation of affect, social dysfunction and delusions [232]. This spectrum of psychopathology is consistent with some of the positive (e.g., delusions and inattention) and negative symptoms (e.g., flat affect and social withdrawal) of schizophrenia. Other reports found correlations of reduced vermal volume with the depression and paranoia sub-scores of the Brief Psychiatric Rating Scale [223] and greater vermis white matter volumes with severity of positive symptoms, thought disorder and impaired verbal logical memory in patients with schizophrenia [250].

Neuropathology of the cerebellar pedunculi is also relevant to functional deficits described in schizophrenia, such as associative learning and the formation of memory traces for the conditioned eye-blink response [240]. Lower rates of eye-blink conditioning, particularly less adaptively timed conditioned response latencies, have been reported in schizophrenia [255]. The current finding of reduced grey matter in vermis and pedunculi suggests cerebellar pathology that is already emerging following the first episode of illness.

There seems to be some overlap in regional grey matter pathology with cannabis use and FES in right lobule III, but there was no confirmation at group level owing to the overall small effects of cannabis use on grey matter pathology. This observation is consistent with previous reports [273] and is supported by the current data from cannabis using FES subjects who do not show evidence of more profound cerebellar neuropathology related to their cannabis use. However, this observation must be regarded as preliminary owing to the small sample size of cannabis using FES patients in our study.

On the other hand, according to a recent meta-analysis [256], subtle grey matter deficits in right lobules III, IV, and V in cannabis users may lead to somatosensory, language, verbal working memory, spatial, and executive function deficits and may also affect processing of emotional information. The current findings of greater cerebellar pathology with severity of cannabis use history suggest more pronounced deficits in these functional domains with an earlier onset of use and more accumulated drug exposure. This interpretation is supported by meta-analytic data from a small number of studies of cannabis users by Grant and colleagues [15]. Cognitive deficits are most likely to occur in the domains of learning and memory, processing speed, and selective attention (see also reviews by [283, 284]). The potential impact on cognition of cannabis use requires further investigation.

Overall, our study is limited by sample size. This reflects our difficulties of recruiting nondrug-using FES and age-matched cannabis using volunteers with no or very little abuse of other illicit drugs or alcohol. Recruitment of solely cannabis using FES patients is most difficult owing to the high levels of multiple substance abuse in this population. On the other hand, our data suggest that cortical pattern matching overcomes some limitations of other morphometric methods by accurately aligning group-averaged cortical MRI data in relatively small cohorts, thus providing the basis to map more accurately potential brain functional deficits onto in vivo atlases of brain pathology.

5. General Discussion

The authors clinical observations of high rates of cannabis use and clinical co-occurrence of adolescent cannabis use amongst schizophrenia sufferers in a mental health and drug and alcohol specialist psychiatric inpatient unit led to the development of the hypotheses explored in the series of studies contained in this thesis. At that time, the scientific literature had strengthened the already known epidemiological observations linking cannabis use to psychosis. Furthermore animal studies had begun to elucidate the physiologic effects of cannabinoids on the endocannabinoid system. These bodies of work suggested putative biological explanations for the psychotogenic effects of cannabis use and in our review we explored the potential impacts of these physiologic effects in the dominant hypotheses of schizophrenia.

At the time of writing this discussion, medicinal cannabis is proposed for legislative approval in some states in Australia and, internationally, where medicinal cannabis is already legislated, there is a movement towards decriminalisation of its recreational use. The work of this thesis furthers the scientific conversation using functional and structural brain imaging to better understand the impact of a history of heavy cannabis use on executive function and cerebellar brain structure in first-episode schizophrenia patients. In Chapter two, the fMRI Tower of London study showed that cannabis users and schizophrenia patients failed to adequately activate the dorsolateral prefrontal cortex. A common working memory impairment particularly evident in cannabis-using first-episode schizophrenia patients was suggested. We then sought to further explore potential contributions to of cannabis use to cognitive and clinical symptoms in first episode schizophrenia.

We hypothesised that as the cerebellum possesses a high density of cannabinoid (CB) type 1 receptors involved in the neuronal diversification of the developing brain, cannabis abuse may interfere with this process during adolescent brain maturation leading to “schizophrenia-like” cerebellar pathology. In Chapter Three,

we undertook to confirm previous observations of changes in cerebellar morphology in schizophrenia, but for the first time we published data in a first-episode schizophrenia cohort. We found no changes in total cerebellar volume and total grey matter volumes in first-episode schizophrenia patients from healthy control subjects. Total cerebellar white matter was increased and total grey to white matter ratios were reduced in the superior vermis, the left lobule VI, right-inferior lobule IX, extending into left lobule IX and bilaterally in the areas of lobuli III, peduncle and left flocculus. Grey matter changes were prominent in right lobuli III and IX, left flocculus and bilateral pedunculi. Consistent with common clinical, cognitive, and pathophysiological signs of established illness, the findings demonstrated cerebellar pathology in first episode schizophrenia.

In Chapter Four, we showed a lifetime dose dependent regional reduction of grey matter in the right cerebellar lobules and a tendency for more profound grey matter reduction in lobule III with younger age at onset of cannabis use. The overall regional grey matter differences in cannabis users were within the normal variability of grey matter distribution. The first-episode schizophrenia subjects had lower total cerebellar grey to white matter ratios and marked grey matter loss in the vermis, pedunculi, flocculi and lobules when compared to pair-wise matched healthy control subjects. The pattern and degree of grey matter loss did however not differ from age-matched first-episode schizophrenia subjects with co-morbid cannabis use. Our findings indicated small dose-dependent effects of juvenile cannabis use on cerebellar neuropathology but no evidence of an additional effect of cannabis use on cerebellar grey matter pathology in first-episode schizophrenia. Aside from its role in motor coordination, as noted in our previous study, the cerebellum is involved in cognitive functions including attention, working memory, verbal learning and sensory discrimination. A disturbed prefronto-thalamo-cerebellar circuit has been proposed to play a role in the pathophysiology of schiophrenia. Our work suggests that there may be an effect in common of cannabis use and schizophrenia on this functional network and associated cognitive

functions, however the effect of the illness and substance use may be may not be combinatory nor can we assert that the effects are synergistic.

We discussed the putative physiological mechanism for the effects of cannabis use on neural systems, brain structure and function in our first chapter. Furthermore, we explored the potential role of the cannabis on the cannabinoid system in the dominant hypotheses of schizophrenia. The work of this thesis has contributed to our understanding of the effects of cannabis use on executive function by expanding the literature in how cannabis effects cortical perfusion whilst undertaking the Tower of London task. Our work adds to this literature by specifically exploring the function of the networks that subserve the Tower of London task and the structural changes associated with cannabis use and schizophrenia in the cerebellum. Exploration of potential structural prefrontal, frontal brain, temporal and parietal changes and associated functional perturbations is the logical natural extension of this work. In addition, the work undertaken by the collaborators of the author exploring mismatch negativity in schizophrenia [280] may be an additional correlate to explore in the study, as it is increasing more important to employ multiple investigative modalities and paradigms to strengthen study design in imaging work. next step of exploration.

We have replicated previous structural brain imaging work that has shown changes in cerebellar structure in schizophrenia. By exploring the functional correlates of these structural changes in future work we will seek to replicate these findings and assess whether there are functional imaging and clinically relevant cognitive correlates of the structural changes we have described in Chapter Four.

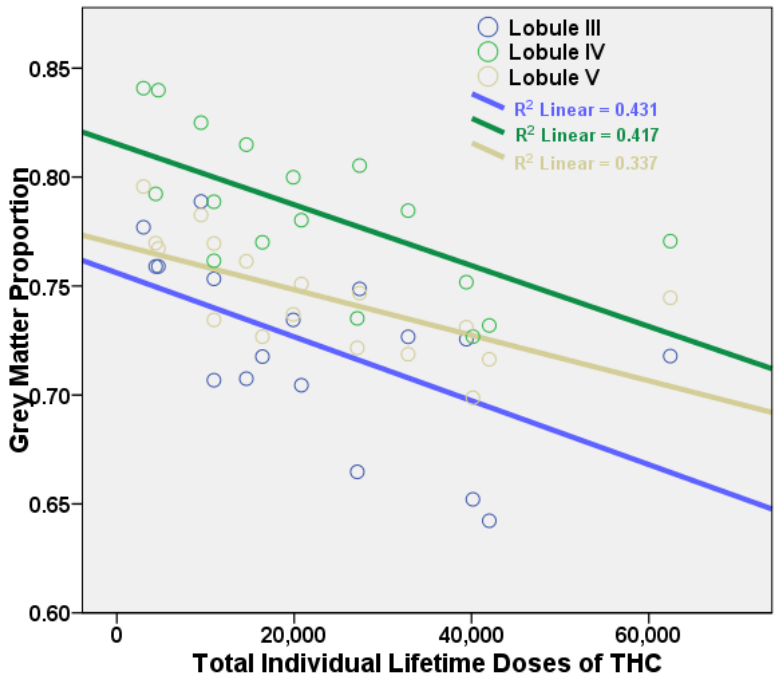
We have acknowledged the challenges of conducting cannabis research in our papers, in particular accurately assessing the dose used. Although controlled for in methodology and tested for effect in analyses, an influence of psychotropic medications, co-morbid psychiatric diagnoses and the effects of alcohol consumption that is known to reduce grey matter volume must be considered as potential validated confounding effects on detected changes in morphology and function. In addition, when we undertook to design the studies there were limited

numbers of cannabis imaging studies of sufficient quality to guide our study design and analysis approach. It is imperative that future studies should consider the need for convergent methodology, a focus on the replication of known findings and with greater methodological rigour as suggested above. Moreover, prospective studies with large sample sizes across the life-span from early adolescence to adulthood will assist in the understanding and delineation of both the development of and reversibility of previously observed changes in brain structure and function and their clinical correlates.

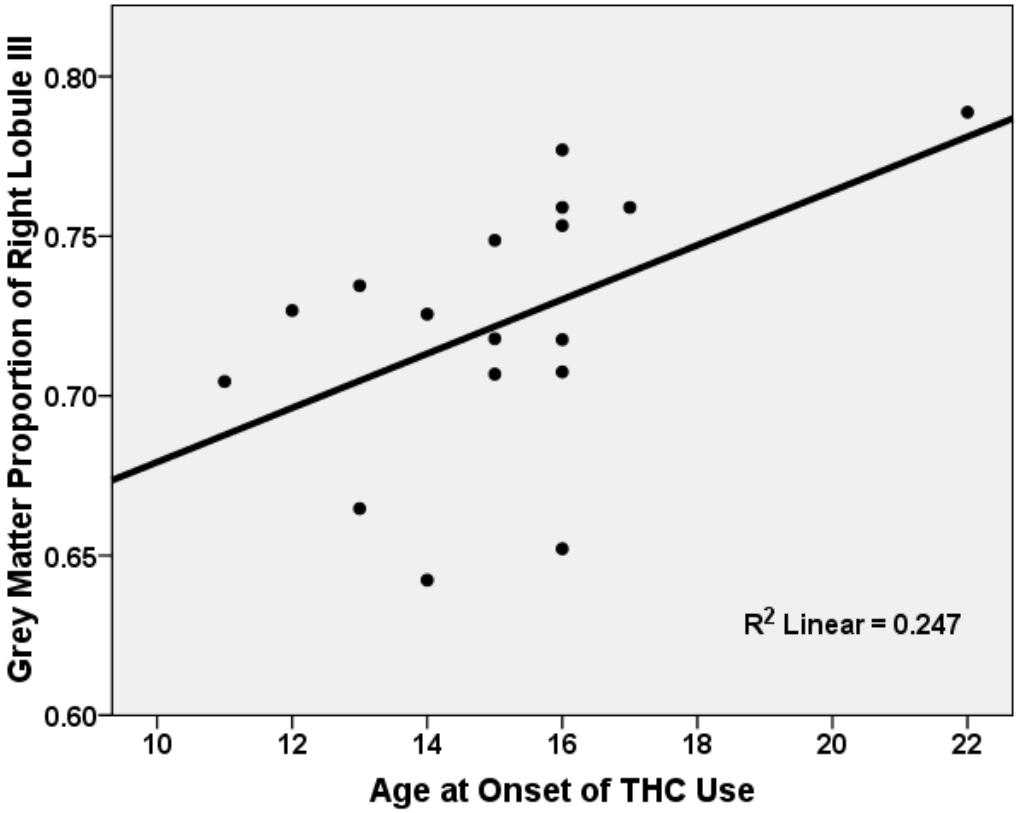
Finally, there is a promising and exciting potential future role for modulating the cannabinoid system by CB1 and CB2 receptor antagonism in the treatment of psychosis. Cannabidiol has a low affinity for CB1 and CB2 receptors and is capable of altering CB1 and CB2 receptor function by antagonising their receptor agonists [285]. Zuardi and colleagues series of small studies suggested that cannabidiol might have antipsychotic properties [286]. More recently, Marcus Leweke and his colleagues [287] have published the first small clinical trial (n=42) comparing cannabidiol to an established antipsychotic medication- amisulpride. They demonstrated that cannabidiol is capable of reducing psychotic symptoms in a manner equally effective to amisulpride but with significantly less impairing side effects. Given the cardiometabolic side-effects of the second generation antipsychotic medications and the extrapyramidal side-effects of the first generation antipsychotics, perhaps modulation of the cannabinoid system may provide an alternative treatment for sufferers of psychosis, with less significant side-effects. It appears that the study and development of our understanding of a potential risk factor for the schizophrenia, cannabis and its constituents, has contributed to the literature that may have opened up new pathways for our understanding of the illness itself and may potentially lead to the development of new improved therapeutic options.

Appendix

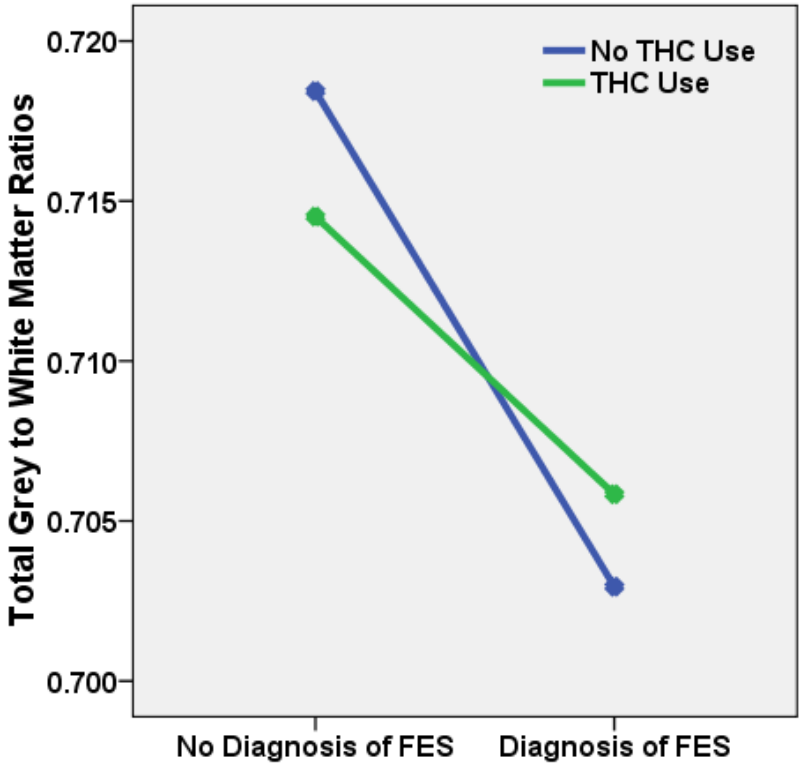
Supplementary Figure S1: Scatterplot of total lifetime doses by grey matter proportion in right lobules III (r_s=-.66, p=0.004), IV (r_s=-.70, p<0.002), and V (r_s=-.78, p<0.001)



Supplementary Figure S2: Scatterplot of age of onset of cannabis use by grey matter proportion in right lobule III (r_s=.51, p<0.04)



Supplementary Figure S3: Boxplot representation of mean ratios of total cerebellar grey to total cerebellar volume for healthy control subjects (CON), cannabis using subjects (THC), first-episode schizophrenia subjects (FES) and cannabis-using first-episode schizophrenia subjects (FES+THC). Main effect of diagnosis of FES: F(1,51)=10.6, p=0.002. There was no main effect for cannabis use nor a significant interaction of cannabis use by diagnosis of schizophrenia



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