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Does cannabis cause lasting brain damage?

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Abstract
Until recently, it was possible to state with some confi - dence that there was no evidence of cannabis-related brain damage in humans. There was some support from the animal literature, but few human studies had been conducted where the findings could not be explained by methodological or other confounding factors. Recent evidence for gross morphological, connectivity and microstructural changes has now emerged that warrants further consideration. If cannabis were found to alter the structural integrity of the brain, then this may assist us to understand the mechanisms by which cannabis triggers psychotic symptoms or overt psychosis in vulnerable individuals.

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Chapter 9

Does cannabis cause lasting brain damage?

Nadia Solowij, Murat Yücel, Valentina Lorenzetti and Dan Lubman

Until recently, it was possible to state with some confidence that there was no evidence of cannabis-related brain damage in humans. There was some support from the animal literature, but few human studies had been conducted where the findings could not be explained by methodological or other confounding factors. Recent evidence for gross morphological, connectivity and microstructural changes has now emerged that warrants further consideration. If cannabis were found to alter the structural integrity of the brain, then this may assist us to understand the mechanisms by which cannabis triggers psychotic symptoms or overt psychosis in vulnerable individuals.

Evidence from animal studies

Cannabinoids, either endogenous or exogenous, possess both neuroprotective and neurotoxic properties (Sarne and Mechoulam, 2005; Kano *et al*., 2009). Cannabinoid-receptor activation induces morphological changes to neurons, such as inhibition of new synapse formation (Kano *et al*., 2009), and at crucial neurodevelopmental stages (prenatal and adolescent), exposure to cannabinoids impacts on neural cell survival and maturation (Chapters 6, 7) (Downer and Campbell, 2010). The role of different cannabinoids in controlling neural-cell survival or death is a complex issue that is influenced by the dose, duration of exposure and route of administration, but also the neural-cell type and its stage of differentiation (Downer and Campbell, 2010). Contradictory hypotheses circulate regarding the doses of Δ^9-tetrahydrocannabinol (THC) that may be neurotoxic or neuroprotective. Some suggest that single high doses of THC are neuroprotective within a limited timeframe, but that low doses are neurotoxic and, with chronic exposure, induce neuronal death (Sarne and Keren, 2004; Tselnicker *et al*, 2007; Sarne and Mechoulam, 2005). However, large doses of THC applied directly to cultured hippocampal neurons, and both high and low doses to cultured cortical neurons, have been shown to cause cell death or significant neurotoxic changes (eg. shrinkage of cell bodies and DNA-strand breaks) characteristic of neuronal apoptosis (Chan *et al*., 1998; Campbell, 2001; Downer *et al*., 2001). Indeed, even a single administration of an ultra-low dose of THC (0.001–0.002 mg/kg) has been shown to result in long-term cognitive impairment (in spatial learning, strategy and working memory) in mice. These defecits persisted for at least 5 months post-injection and were associated with activation of extracellular-regulated kinase (ERK) in the cerebellum and hippocampus (Tselnicker *et al*., 2007; Amal *et al*., 2010). The authors suggested that low THC concentration is the main determinant of long-lasting neuronal effects following chronic exposure to cannabinoids, due to their slow clearance and accumulation (Amal *et al*., 2010).

A study of cannabinoid application in vitro showed that THC appears to accumulate primarily in neurons and that transformation to its metabolite, THC-COOH, depends on the presence of glia (Monnet-Tschudi *et al*., 2008). The authors suggested that the adverse effects of cannabinoids on the brain may occur through a combination of pathways involving cannabinoid receptor activation, accumulation of cannabinoids and their metabolites and upregulation of neuroinflammatory cytokines. Given the dependence on glia for metabolism of THC, if white-matter aberrations develop in cannabis users (as discussed further below), more THC could potentially accumulate in neurons, causing toxicity.

Studies of chronic cannabinoid administration to animals have demonstrated cognitive impairment associated with specific neurochemical, transmission and cell firing alterations, particularly in the hippocampus, but also the prefrontal cortex (PFC), similar to impairment

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induced by lesions or transient inactivation (Egerton *et al*., 2006). Chronic administration of THC to rats and primates has been shown to result in dose-dependent neurotoxic changes in brain regions that are rich in cannabinoid receptors. Specifically, THC-induced neurotoxic effects are prominent within the hippocampus (Heath *et al*., 1980; Scallet *et al*., 1987; Landfield *et al*., 1988; Chan *et al*., 1998; Lawston *et al*., 2000), amygdala (Heath *et al*., 1980), septum (Harper *et al*., 1977; Myers and Heath., 1979) and cerebral cortex (Harper *et al*., 1977; Downer *et al*., 2001). These neurotoxic effects include shrinkage of neural cell nuclei and bodies (Heath *et al*., 1980; Scallet *et al*., 1987) and reductions in pyramidal cell density (Lawston *et al*., 2000), dendritic length (Landfield *et al*., 1988) and number of synapses (Heath *et al*., 1980). Some of these studies have emulated chronic-use patterns seen in humans: for example, in the study by Landfield and colleagues (1988) THC was administered to rats five times a week for 8 months, representing approximately 30% of the rats' lifespan.

Cannabis use in humans typically commences during adolescence and young adulthood, a crucial period of neurodevelopment (see Chapter 7). Neuromaturational changes primarily occur within PFC and limbic circuits, and include progressive and regressive changes such as myelination and synaptic pruning, neurogenesis and apoptosis, axonal growth and sprouting, dendritic arborization and retraction, synaptogenesis and synapse elimination, alongside the maturation of multiple neurotransmitter systems (Schneider, 2008; Realini *et al*., 2009). The endocannabinoid (eCB) system (ECS) is crucially involved in these developmental processes (Harkany *et al*., 2008; Schneider, 2008; Realini *et al*., 2009) that are thought to be essential for the acquisition of adult cognition, decision-making and social behaviors. As such, exposure to THC during adolescence may perturb neurodevelopmental processes with potential long lasting consequences.

As discussed in detail in Chapter 7, a number of studies have examined the impact of THC administration during adolescence on the adult brain. For instance, Rubino and colleagues (2009a) administered THC twice daily to adolescent rats for 10 days and then left them undisturbed until adulthood, at which point they assessed learning and memory capacities, as well as their underlying neural substrates. Deficits in spatial memory were evident in the pretreated rats and were accompanied by, and correlated with, significantly lower total dendritic length and number, reduced spine density and decreases in astroglial markers, protein expression and N-methyl-D-aspartate receptor levels within the hippocampus. Thus, adolescent exposure to THC resulted in long-lasting alterations to the structural and functional plasticity of both neurons and glia, with a reduction in synaptic contacts and/or less efficient synaptic connections throughout the hippocampus. In other studies, these authors found significant gender-related THC neurotoxic effects, demonstrating that CB1 receptor density and G-protein coupling were significantly reduced in the amygdala, ventral tegmental area and nucleus accumbens in female rats, but only in the amygdala and hippocampus of male rats, accompanied by different behavioral profiles (Rubino *et al*., 2008). Further, spatial working memory impairment was similar between genders but was underpinned by hippocampal alterations in males, in contrast to PFC alterations in females (Rubino *et al*., 2009b). This work supports a growing literature demonstrating sex differences in adulthood in animals chronically administered THC during adolescence, as well as alterations within circuits underlying emotional processing (Realini *et al*., 2009).

There is also growing evidence of differential responses to cannabis during adolescence compared with adulthood. Quinn *et al*. (2008) found that repeated exposure to THC was less behaviorally aversive for adolescent compared with adult rats, but caused greater persistent memory deficits and hippocampal structural and functional alterations. Altered protein expression in the hippocampus was observed in both adult and adolescent rats, but adolescent rats showed a greater number of altered proteins related to oxidative stress, mitochondrial and metabolic function, cell proliferation and repair, and cytoskeletal structure and signaling. Further recent research on neurotransmitter system functionality and cannabinoid receptor changes following chronic exposure to cannabinoids suggests that the adolescent brain does not compensate for the biological changes in response to cannabis exposure in the same way as the adult brain (Dalton and Zavitsanou, 2010; Zavitsanou *et al*., 2010). The ECS also appears to be altered by exposure to THC during early, middle and late adolescence (Ellgren *et al*., 2008). In a rat study, intermittent exposure to THC (a pattern of use common among teenagers) was found to reverse the normal proportional ratio of eCBs (anandamide and 2-arachidonoyl glycerol [2-AG]) in the PFC and nucleus accumbens. These studies support the notion that THC effects on neural integrity may depend on different developmental stages of exposure.

Thus, evidence from preclinical research has identified neurotoxic, morphological and microstructural alterations to the brain in vitro and, when animals are acutely or chronically exposed to cannabinoids, at doses relevant to human use. With discrepant results concerning the neurotoxicity of low and high doses, and accumulation of cannabinoids, further research must reconcile dose-effects in vitro versus in vivo, and consider the various cannabinoids that human users expose themselves to. Some of these have been shown to have differential properties and opposing effects in humans (eg. THC versus cannabidiol; see Chapter 1). As such, animal research could examine each of these in isolation and in combination, and further elucidate their impact on the developing brain.

Evidence from human studies

Adult chronic cannabis users

Findings of persistent alteration of brain function or cognitive impairment in human cannabis users (as reviewed in Chapter 8), together with the animal work discussed above, support the notion that long-term cannabis use may result in morphological alterations of brain structures that subserve attention, learning, memory, executive functions and emotional processes (such as the prefrontal and temporal cortices). To date, findings from structural neuroimaging studies of long-term cannabis users have been contradictory, with evidence for both the presence and absence of morphological changes in specific brain regions (DeLisi, 2008; Solowij *et al*., 2009; Lorenzetti *et al*., 2010; Martin-Santos *et al*., 2010). However, a number of variables, such as demographic, clinical, genetic and drug-use factors are likely to mediate the relationship between cannabis use and brain structural alterations.

A recent review (Lorenzetti *et al*., 2010) identified only 13 structural neuroimaging studies where the primary substance used was cannabis and major psychopathologies were excluded. The main imaging modality utilized was magnetic resonance imaging (MRI) (eight studies), with three studies employing computed tomography (CT) and two early studies using pneumo-encephalography and echo-encephalography, respectively. The MRI studies used either a region-of-interest approach (six studies) or voxel-based morphometry (VBM; two studies). No significant differences were found in any of the studies on global measures of brain volume. More specific regional brain analyses demonstrated evidence of structural brain abnormalities, but these were not consistent across studies.

Six studies reported specific regional structural alterations in regular cannabis users (Campbell *et al*., 1971; Block *et al*., 2000; Wilson *et al*., 2000; Matochik *et al*., 2005; Medina *et al*., 2007b; Yücel *et al*., 2008), while the remaining seven studies found no significant volumetric differences between users and controls (Stefanis, 1976; Co *et al*., 1977; Kuehnle *et al*., 1977; Hannerz and Hindmarsh, 1983; Jager *et al*., 2007; Medina *et al*., 2007a; Tzilos *et al*., 2005). Alterations in hippocampal or parahippocampal volumes were the most consistently reported findings, but the nature of the findings were still mixed. Hippocampal volumes in cannabis users were found to be smaller (Matochik *et al*., 2005; Yücel *et al*., 2008), larger (Medina *et al*., 2007b), or no different to controls (Block *et al*., 2000; Wilson *et al*., 2000; Medina *et al*., 2007a). Of three studies that examined parahippocampal volume, two reported no change (Jager *et al*., 2007; Tzilos *et al*., 2005), while one found an alteration in grey and white matter composition (Matochik *et al*., 2005). Two studies examined amygdala volumes, with one reporting reduced volume (Yücel *et al*., 2008) and the other no change (Wilson *et al*., 2000). Finally, there were a number of brain regions that were investigated only within a single study, with significant between-group differences found for the precentral gyrus, thalamus, parietal lobule, fusiform gyrus, lentiform nucleus and pons (Matochik *et al*., 2005), but not for the cerebellum (Block *et al*., 2000). While few studies have specifically examined white-matter volume, we recently identified significant cerebellar white matter reduction in adult long-term very heavy cannabis users (Solowij *et al*., 2011).

Dose and duration of cannabis exposure may differentiate between those studies that did or did not find volumetric differences between users and controls. For example, in our study (Yücel *et al*., 2008) the cannabis users had a similar exposure to that of Landfield *et al*.'s (1988) rodent study (cited above). Both of these studies found significant dose-related reductions in hippocampal volume. The cannabis users within our study had the most extensive exposure to cannabis of all the studies of human cannabis users (near daily use for a mean 19.7 years, range 10–32 years), and the most striking findings. We reported a 12% reduction bilaterally in hippocampal volumes, as well as an approximate 7% reduction in bilateral amygdala volumes (Yücel *et al*., 2008), and a 24% reduction in cerebellar white matter (Solowij *et al*., 2011). The reduction of left hippocampal

volume was of a similar magnitude to that observed in schizophrenia, was dose-related and was associated with subclinical psychotic symptoms, even though our sample was carefully screened for DSM-IV psychotic disorders.

One other study with a similar mean duration of use (mean 22.6 years, range 12–33 years) to the sample in our study, reported no brain alterations, but the minimum duration of *daily* use in that sample was only one year (Tzilos *et al*., 2005). In contrast, the minimum duration of *near daily* use in our study was 10 years. A further key difference between the Tzilos *et al.* (2005) study and ours was in the estimated episodes of use, and hence the cumulative dose of exposure to cannabis. Tzilos *et al*.'s sample reported an average of 20 100 lifetime episodes of use. Our sample had an average 62 000 estimated episodes of use over the lifetime. Thus, despite a similar mean duration of use, our cannabis users used more than three times as much cannabis, which may be the crucial factor in explaining our finding of a dose–response relationship between hippocampal volume and cumulative cannabis use. In addition, Tzilos *et al.* (2005) acquired their images at a lower field strength and with a coarser spatial resolution (1.5 T with 3-mm-thick slices vs. 3 T with 1-mm-thick slices in our study), an important consideration given the small size and boundary definition of the brain structures investigated. Moreover, the region of interest measured in their study was less specific to the hippocampus relative to ours because they also included the parahippocampal gyrus (ours was restricted to the hippocampus itself using well-defined boundaries).

A general trend for an inverse relationship between indices of cannabis use and hippocampal or parahippocampal volume appears to exist in other studies. Aside from our own study, samples with greater cannabis exposure demonstrated reductions in hippocampal or parahippocampal volumes (Matochik *et al*., 2005), whereas samples with a lower quantity or frequency of cannabis use exhibited no change (Block *et al*., 2000; Wilson *et al*., 2000; Jager *et al*., 2007; Medina *et al*., 2007a; Tzilos *et al*., 2005), or even volumetric increases (Medina *et al*. 2007b). Studies of heavy cannabis users (Matochik *et al*., 2005; Yücel *et al*., 2008) were more likely to detect regional abnormalities than those of lighter cannabis users. Greater brain alterations with an earlier age of onset of cannabis use have been reported in some studies (Wilson *et al*., 2000), but not others (Matochik *et al*., 2005; Tzilos *et al*., 2005), but this aspect of human cannabis use remains underinvestigated.

Several recent studies have examined the integrity of white matter fiber tracts in cannabis users using diffusion tensor imaging (DTI), including studies of adolescent users (reported below). A pilot study in ten heavy cannabis users demonstrated trends toward both increased mean diffusivity and lower fractional anisotropy in the anterior cingulate cortex (Gruber and Yurgelun-Todd, 2005). Another study of heavy users found significantly increased mean diffusivity in the anterior region of the corpus callosum, where white matter passes between the prefrontal lobes (Arnone *et al*., 2008). The data suggest impaired structural integrity of the corpus callosum fiber tracts with prolonged cannabis exposure, particularly as the authors reported an association with duration of cannabis use within the sample. White matter tractography investigations in cannabis users are only at a preliminary stage of investigation and hold much promise for the future.

A post-mortem study of cannabinoid receptor density and integrity in human brains found that the receptor becomes hypofunctional with chronic cannabis use (Villares, 2007). Downregulation was observed in the hippocampus, basal ganglia and mesencephalon of chronic users, and reduced binding levels were accompanied by parallel decreases in mRNA levels. These findings suggest that the primary effect of chronic exposure was on the CB1 receptor gene rather than on the receptor protein. Evidence of diminished neuronal and axonal integrity in the dorsolateral prefrontal cortex has been indicated by magnetic resonance spectroscopic markers of metabolism (NAA/tCr ratio) (Hermann *et al*., 2007). Dose-related changes in this study were also found in the anterior cingulate and putamen/globus pallidum, but not in the hippocampus. Acute and chronic exposure to cannabis in humans has also been associated with reduced serum concentrations of neurotrophins, including nerve growth factor (Angelucci *et al*., 2008) and brain derived neurotrophic factor (BDNF) (D'Souza *et al*., 2009).

Thus, there is growing evidence for alterations to the structural integrity of the brain as a result of chronic cannabis exposure in adult users. This includes gross structural anatomical studies of long term and heavy users, as well as more refined studies of white matter and connectivity, and neurotoxic markers in vivo.

Adolescent and young-adult cannabis users

An increasing number of studies have investigated brain morphology in adolescent cannabis users or in adults who started using cannabis at a young age. A

study of adult users reported that early onset cannabis users (before age 17 years) had smaller whole brain volumes, lower percent cortical grey matter, higher percent white matter and increased cerebral blood flow compared with later onset users (Wilson *et al*., 2000).

The two studies by Medina and colleagues discussed above were of adolescents, one reporting larger hippocampal volumes in users (Medina *et al*., 2007b), while the other found no volumetric differences from controls (Medina *et al*., 2007a). Medina *et al.* (2007a) also found an association between whole brain white matter volume and depressive symptoms in young adult cannabis users. While a DTI study of young adults who had at least one year of daily or several times/week cannabis during adolescence, found no evidence of pathological white matter integrity differences between users and controls, it identified several regions of apparently greater integrity among users (DeLisi *et al*., 2006). However, a solid body of evidence for pathology in white matter tracts within the corpus callosum and various fronto-temporal, occipito-frontal and posterior connections that develop during adolescence, has come from other recent DTI studies of young adult (Arnone *et al*., 2008; Allin *et al*., 2009) and adolescent (Ashtari *et al*., 2009; Bava *et al*., 2009; Yücel *et al*., 2011) cannabis users, as well as adolescents with substance use disorders (primarily cannabis) (Thatcher *et al*., 2010). Abnormalities in this latter study were greater in females than in males. The results from these studies overall suggest that cannabis use, particularly during adolescence, may affect the trajectory of normal brain maturation resulting in white matter aberrations, which may underlie compromised cognitive processing and may even underpin the propensity for cannabis to cause psychosis (Allin *et al*., 2009; Solowij *et al*., 2011).

Interestingly, Jacobus *et al*. (2009) reported greater white matter integrity alterations in several brain regions in adolescent binge drinkers than in adolescent heavy cannabis users who were also binge drinkers. The latter group differed from controls in three regions, while alcohol only users differed in eight regions. The data suggest subtle white-matter-tissue microstructural abnormalities reflecting poor tract coherence and organization, but not tissue loss or demyelination, and imply interactive effects of cannabis and alcohol or a possible neuroprotective role of cannabis in binge drinking. In a further investigation of cognitive function in relation to white matter integrity, these same authors found that reduced white matter integrity in temporal regions in cannabis and alcohol using adolescents was associated with poor performance in attention, working memory and speed of processing tasks (Bava *et al*., 2010). Higher integrity of white matter fiber tracts in the cannabis users relative to controls (interpreted as a neurodevelopmental compensatory mechanism in response to exposure to cannabis) was associated with better performance, except for in one anterior region where higher integrity was associated with poorer contextual verbal memory performance. The interactive effects of cannabis and alcohol should be further investigated, particularly as they are frequently used together by adolescents.

Altered cortical gyrification in the frontal lobe and abnormal age-related changes to gyrification and cortical thickness have also recently been reported in adolescent and young adult users (Mata *et al*., 2010). Cannabis users showed bilaterally decreased concavity of the sulci (i.e. greater flattening) in frontal, temporal and parietal lobes, and thinner sulci in the right frontal lobe, in the absence of global brain structural differences between users and controls. Abnormal cortical gyrification may reflect abnormal neurodevelopment or neurodegeneration. A lack of normal association between these measures and increasing age in the cannabis users, together with a lack of observed associations with specific cannabis-use parameters led the authors to speculate that cannabis use during adolescence or young adulthood might prematurely alter cortical gyrification toward patterns usually seen at a later age.

Further specific investigations of brain structure and function are clearly warranted in adolescent cannabis-using samples to verify whether cannabis has specific and/or more detrimental effects than in adult users; whether there are age-of-onset-dependent and gender effects; and whether there is a progression of brain morphological abnormalities with continued cannabis use, or reversal with abstinence.

Patients with psychosis

Since brain structural changes are evident in patients with schizophrenia, and there is mounting evidence for similar changes in association with heavy cannabis use, it is possible that cannabis may exert greater adverse effects on brain morphology when the brain is already compromised. Indeed, this is most likely to occur in brain regions known to be altered in both heavy cannabis users and patients with schizophrenia (e.g., hippocampus). In line with this, a number of recent studies have investigated brain morphology in patients with

schizophrenia or early psychosis and comorbid cannabis use.

No differences in brain structure between patients with established schizophrenia who did and did not use cannabis were reported by Cahn *et al.* (2004), while Potvin *et al.* (2007) found increased striatal grey matter densities in schizophrenia patients with comorbid substance-use disorders (primarily cannabis). In first-episode psychosis patients who use cannabis, decreased grey matter volumes of the anterior cingulate (Szeszko *et al.*, 2007), right posterior cingulate cortex and left hippocampus (Bangalore *et al.*, 2008) were reported relative to their non-using counterparts and to healthy controls. Trends toward smaller left and right cerebellar volumes were also apparent (Bangalore *et al.*, 2008). Rais and colleagues (2008) reported greater lateral and third ventricle enlargements and more pronounced total cerebral grey matter volume reduction over 5 years in first-episode schizophrenia patients who used cannabis compared with those who did not, as well as in comparison with healthy controls (2.67% and 5.09% reduction, respectively). The results were suggested to explain some of the detrimental effects of cannabis use in patients with schizophrenia. We have recently reported hippocampal shape alteration (Solowij *et al.*, 2010) and an almost 30% loss of cerebellar white matter relative to healthy controls in patients with schizophrenia and extensive cannabis use histories (Solowij *et al.*, 2011). Finally, Dekker *et al.* (2010) found that the age of onset of cannabis use (before age 15 years versus age 17 years or later) had no bearing on white matter integrity of the corpus callosum in a sample of young adults with recent onset schizophrenia, while cannabis-naïve patients showed greater abnormalities. These results support the notion that cannabis-using patients may represent a group who developed psychosis in part at least as a consequence of their cannabis use (Dekker *et al.*, 2010; Yücel *et al.*, 2010). Clearly, the impact of cannabis use on brain function and structure in schizophrenia also warrants further investigation.

What might be the implications of structural brain changes in cannabis users?

It is often assumed that alterations in the morphology of the brain may underlie impaired cognition and may indicate neural substrates of risk for the development of psychosis, but there are limited data to support these notions. The interrelationships between cognition and psychopathology, and indeed between brain structure and function, are complex.

Few structural brain imaging studies of cannabis users have specifically examined the relationship between brain volumes and cognitive performance measures and most of those that did found no associations (Tzilos *et al.*, 2005; Jager *et al.*, 2007; Medina *et al.*, 2007b; Yücel *et al.*, 2008) or isolated relationships (Medina *et al.*, 2007a; Solowij *et al.*, 2008). An exception to this was the finding of poor white-matter structural integrity being related to poorer cognitive performance in cannabis and alcohol-using adolescents (Bava *et al.*, 2010). The lack of association in most studies might be interpreted as aberrant associations between brain structure and function, as discussed elsewhere (Solowij *et al.*, 2009).

The growing literature reporting an association between cannabis use and the development of psychopathology, including both psychotic and depressive symptoms, has searched for mediators of risk such as genes (eg. *COMT*; Caspi *et al.*, 2005), but associations between the development of psychotic or depressive symptoms and brain changes in cannabis users have not been rigorously investigated. We reported an association between smaller left hippocampal volume in cannabis users and subclinical positive psychotic symptoms as measured by the Scale for the Assessment of Positive Symptoms (SAPS) (Yücel *et al.*, 2008). Positive symptom scores were also correlated with cumulative cannabis exposure. The cannabis users in our sample were carefully screened for DSM-IV Psychotic Disorders, had never been diagnosed with Major Depressive Disorder and had never sought treatment for any psychological disorders. Yet the majority of the sample endorsed beliefs (scores on the SAPS ranged from Questionable to Mild) concerning ideas of persecution, reference, mind reading, sin and/or guilt, while some displayed bizarre clothing/appearance or reported bizarre social/sexual behavior. Smaller left hippocampal volume was also significantly correlated with higher scores on the paranoid subscale of the Brief Symptom Inventory (BSI) (unpublished data). Negative symptoms were elevated in the cannabis users but were unrelated to hippocampal volumes. Depressive symptoms, which were also elevated, did not correlate with volumetric measures of any brain region, and the relationship between left hippocampal volume and cumulative exposure to cannabis remained significant

after controlling for depressive symptoms. An association between depression and hippocampal volume is seen in the more persistent forms of Major Depressive Disorder (eg. MacQueen *et al*., 2005; Lorenzetti *et al*., 2009), which does not apply to our sample. One other study has reported an association between overall brain white-matter volume and depressive symptoms in adolescent/young adult cannabis users without diagnosable mood disorders (Medina *et al*., 2007b).

Subclinical positive psychotic symptom scores in the heavy cannabis users of our sample correlated with spatial span errors, but no other associations between cognitive measures and symptoms were observed (Solowij *et al*., 2008). Skosnik and colleagues (2001, 2006, 2008) have found associations between cognitive (eg. poor negative priming) and psychophysiological measures (e.g. P300 to affective stimuli; 20 Hz neural synchrony), and higher scores on the Schizotypal Personality Questionnaire, on which cannabis users generally obtained high positive-syndrome scores.

Acute administration of THC to healthy volunteers and patients with schizophrenia induces both cognitive impairment and transient positive and negative symptoms (Chapter 18; D'Souza *et al*., 2004, 2005; Koethe *et al*., 2006), and sensitivity to the psychosis-inducing *and* cognitive-impairing effects of cannabis may be genetically mediated (Chapter 12, Henquet *et al*., 2006). In patients with schizophrenia, associations between positive psychotic symptoms and memory deficits, and volumetric measures of the hippocampus, the superior temporal gyrus, and the temporal lobe in general, have been demonstrated, as well as between negative symptoms, executive function and prefrontal cortical measures (Antonova *et al*., 2005; Gur *et al*., 2007; Nestor *et al*., 2007; Hurlemann *et al*., 2008) and in particular, in relation to white matter structural integrity (Szeszko *et al*., 2008). This suggests that further investigation of brain structural changes in cannabis users, in relation to symptoms and cognition, is warranted.

A crucial question is the extent to which interrelated structural-functional aberrations involving the hippocampus, prefrontal regions or indeed other brain structures in cannabis users, might reflect a vulnerability to schizophrenia. Our own findings suggest that long-term exposure to cannabis constitutes a vulnerability to psychopathology by disrupting the structural integrity of brain regions that are also involved in psychotic (and affective) disorders. We propose that long-term heavy cannabis use leads to structural brain changes and associated deleterious functional (cognitive and mental health) sequelae that resemble aspects of schizophrenia. These changes may occur not only in individuals who are vulnerable to the development of such disorders, but also in nonvulnerable individuals if cannabis is used heavily for prolonged periods or commences during crucial neurodevelopment periods such as early adolescence.

Conclusions

Strong evidence for cumulative, sometimes dose-related, neuronal damage or microstructural alterations following chronic exposure to cannabinoids (largely THC) comes from the animal literature. While previous research failed to identify structural brain abnormalities in human cannabis users, more recent studies using high-resolution imaging techniques, combined with more robust delineations of specific brain regions in very heavy cannabis users, have revealed evidence of dose-related alterations, mostly in the hippocampal and parahippocampal regions. Our own findings of significant hippocampal and amygdala volume loss in cannabis users suggest potential toxicity due to cumulative exposure to large doses of cannabis over many years. However, the structural neuroimaging studies of cannabis users have so far focused on a narrow range of brain regions. Cannabis use, particularly during early adolescence, may affect the morphology of other cortical (e.g. PFC) and subcortical (e.g. striatum) brain areas, where cannabinoid receptors are heavily concentrated. Hippocampal changes accord with hippocampal functional alterations in functional imaging studies, which together with evidence of aberrations from spectroscopic and DTI studies, implicate the PFC. Evidence for damage to white-matter integrity in cannabis users implicates neural circuitry across multiple regions. Differences in the methods of measurement used and the brain regions investigated and small sample sizes of varying age and exposure to cannabis, may have contributed to the heterogeneity of findings across human studies overall.

While the evidence is only beginning to accumulate from a small number of studies that have used rigorous methods to investigate structural brain alterations in cannabis users, it seems that long-term heavy cannabis use can result in brain pathophysiological and functional changes that resemble aspects of schizophrenia. The data suggest that such alterations are likely to occur when cannabis is used very heavily over a prolonged period and typically involve medial

temporal lobe structures. The cumulative evidence for neurocognitive dysfunction similar to that seen in schizophrenia and the development of subclinical psychotic symptoms in cannabis users, combines with the limited data from structural neuroimaging studies to support our proposition that chronic cannabis use may result in schizophrenia-like changes in brain structure and function. This is further supported by evidence that long-term exposure to cannabis may result in lasting dysfunction of the endogenous cannabinoid system, as well as alterations in the functionality of a number of neurotransmitter systems – changes that resemble schizophrenia-like conditions in the brain (see Solowij *et al*., 2009). There may be multiple moderators or mediators of adverse sequelae from long-term heavy cannabis use, including genetic variation, gender, environmental factors and early neurodevelopmental insults and stress, that interact with cumulative exposure to high-dose cannabis use to produce schizophrenia-like sequelae. A crucial factor is in determining the parameters of cannabis use that lead to these structural and functional alterations in individuals who are, compared with those who are not, at high risk for the development of neuropsychiatric disorders, at various neurodevelopmental periods, and identifying the protective mechanisms that prevent the onset of such potentially devastating disorders.

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