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Effects of frequent cannabis use on hippocampal activity during an associative memory task

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Abstract Interest is growing in the neurotoxic potential of cannabis on human brain function. We studied non-acute effects of frequent cannabis use on hippocampus-dependent associative memory, investigated with functional Magnetic Resonance Imaging (fMRI) in 20 frequent cannabis users and 20 non-users matched for age, gender and IQ. Structural changes in the (para)hippocampal region were measured using voxel-based morphometry (VBM). Cannabis users displayed lower activation than non-users in brain regions involved in associative learning, particularly in the (para)hippocampal regions and the right dorsolateral prefrontal cortex, despite normal performance. VBM-analysis of the (para)hippocampal regions revealed no differences in brain tissue composition between cannabis users and non-users. No relation was found between (para)hippocampal tissue composition and the magnitude of brain activity in the (para)hippocampal area. Therefore, lower brain activation may not signify neurocognitive impairment, but could be the expression of a non-cognitive variable related to frequent cannabis use, for example changes in cerebral perfusion or differences in vigilance.

1. Introduction

Cannabis[1] is the most popular of all illicit drugs (UNODC, 2005; TNDM, 2004). Although cannabis has long been considered to be more benign than other drugs, interest in the neurotoxic potential of cannabis on the brain and brain function is growing. Until now, there is very limited proof for structural brain abnormalities in frequent cannabis users. Although *ex vivo* studies in rat hippocampal neurons in culture have revealed delta9-THC-induced cell death with shrinkage of neurons and DNA fragmentation (Chan et al., 1998; Lawston et al., 2000), in humans the picture is less clear. Several studies failed to demonstrate morphometric changes of the brain as a whole and the hippocampus in

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[1] With the term 'cannabis' we refer to substances with as major psychoactive compound delta 9-tetrahydrocannabinol (THC), i.e. marijuana, weed etcetera.

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particular, in long-term cannabis users (Block et al., 2000; Tzilos et al., 2005). On the other hand, recent findings from a voxel-based morphometry (VBM) study suggest that cannabis is neurotoxic to human hippocampal neurons, with lower gray matter tissue densities bilaterally in the hippocampal regions in frequent cannabis users compared to non-users, and indications for subtle structural changes in other brain regions as well (Matochik et al., 2005). Evidence for functional, i.e. cognitive abnormalities after extended and heavy use of cannabis is somewhat stronger. Acute effects include impairments of perceptual-motor and cognitive tasks, especially memory and learning (Block and Ghoneim, 1993; Curran et al., 2002; Fletcher et al., 1996; O'Leary et al., 2002). Effects of long-term use of cannabis are less consistent: some studies did not find proof for persistent effects of cannabis use (Kalant, 2004; Lyketsos et al., 1999), while others reported subtle impairments of memory and learning, executive functions and attention (Block et al., 2002; Bolla et al., 2002; Eldreth et al., 2004; Grant et al., 2003; Pope, 2002; Pope et al., 2002; Solowij et al., 2002). It is important to note that only few studies investigated persistent, long-term effects of cannabis use on cognition with adequately long monitored abstinence periods (approximately one month) (Bolla et al., 2002; Eldreth et al., 2004; Pope, 2002; Pope et al., 2002). In other studies abstinence periods were much shorter (17–28 h), in which case reported effects were likely to reflect sub-acute, possibly transitory effects of cannabis use on cognition (Block et al., 2002; Solowij et al., 2002).

It has been suggested that cannabis affects certain aspects of memory more profoundly than others. Evidence for this hypothesis comes from a study where infrequent cannabis users showed impaired episodic memory and learning in a dose-dependent manner, whereas implicit memory and working memory were unaffected (Curran et al., 2002). The question arises whether impaired episodic memory is accompanied by altered brain function, and if so, whether specific brain regions are involved. Differential involvement of brain regions in different types of memory is well documented based on functional neuroimaging studies (for a review see Cabeza et al., 2002; Cabeza and Nyberg, 2000). For example, prefrontal areas play an important role in working memory (D'Esposito et al., 1995), whereas formation and retrieval of episodic memory, specifically associative memory, rely more heavily on temporal brain regions such as the hippocampus and the parahippocampal gyrus (Desgranges et al., 1998; Eichenbaum et al., 1996; Henke et al., 1997). Interestingly, the highest densities of cannabinoid receptors (CB1) are found in the hippocampus, cerebellum and striatum (Ameri, 1999; Iversen, 2003). Therefore, the hippocampal region can be regarded as the most likely candidate region for the observed impairments in memory and learning in heavy cannabis users. Results from imaging studies using a cognitive task challenge indeed indicate sustained changes in regional cerebral blood flow (rCBF) and neurophysiological abnormalities in (para)hippocampal regions (among other brain regions) in frequent cannabis users (Block et al., 2002; Eldreth et al., 2004; Jacobsen et al., 2004). However, these previous studies have some methodological limitations. First, findings may reflect sub-acute effects from lingering cannabis intoxication (Block et al., 2002). Second, the choice for a particular cognitive paradigm was motivated by the cognitive function under investigation, i.e. working memory (Jacobsen et al., 2004) and executive functioning (Eldreth et al., 2004); functions in which the hippocampus is not necessarily involved. To illustrate, in a recent study from our own laboratory on the long-term effects of cannabis use on working memory and attention, we found evidence for involvement of the dorsolateral prefrontal and the anterior cingulate cortex (consistent with many other functional Magnetic Resonance Imaging (fMRI) studies on working memory and attention), but no evidence for hippocampal involvement (Jager et al., 2006). It is clear that examination of sustained effects of cannabis on (para)hippocampal function requires a paradigm that activates this region. An appropriate paradigm for this is one that addresses associative memory, which has been shown previously to activate the hippocampus (Eichenbaum et al., 1996; Henke et al., 1997).

The purpose of the current study is to provide a comprehensive assessment of potential sustained effects of cannabis on hippocampus-dependent associative memory function. Behavioral, functional and structural measures of the (para)hippocampal region are acquired from frequent, but abstinent cannabis users and matched controls, using fMRI. The primary objective is to answer the following research questions. First, is performance on a specific, hippocampal-dependent associative memory task affected in frequent, but abstinent cannabis users compared to non-using controls? Second, are there differences in brain activity in the network of regions involved in associative memory between these users and the controls? If this is the case, then which area(s) exhibit abnormal function, and is the hippocampus involved? Third, is there evidence for structural abnormalities in the (para) hippocampal brain region in frequent cannabis users.

2. Experimental procedures

2.1. Subjects

Twenty frequent cannabis users (lifetime use: median 1,900 joints; range 675–10,150 joints) were compared to twenty (almost) drug naive healthy control subjects (lifetime use: median 0 joints; range 0–30 joints). The groups were matched for age, gender and verbal IQ. Half of the subjects (10 users, 10 controls) were selected from a prospective study on ecstasy neurotoxicity (De Win et al., 2005) and a study on the long-term effects of cannabis use on working memory and attention (Jager et al., 2006). The remaining subjects were recruited through advertisements in newspapers or Internet, at locations where cannabis is sold, at university colleges and through word of mouth. Substance and alcohol use was assessed by self-report questionnaires and the Substance Abuse Scales of the Mini International Neuropsychiatric Interview for DSM-IV clinical disorders (M.I.N.I: Translated Dutch Version 5.0.0, (Van Vliet et al., 2000). Verbal intelligence was estimated using the Dutch Adult Reading Test (DART), the Dutch version of the National Adult Reading Test (Nelson, 1991). Inclusion criteria were (1) right-handedness, (2) age between 18 and 35 years, (3) estimated lifetime use of 500 joints or more for the cannabis users, and (4) willingness to abstain from cannabis and alcohol (all subjects) for at least 7 days prior to testing. Compliance was checked by urine drug screening (enzyme-multiplied immunoassay for amphetamine, ecstasy, opiates, cocaine, benzodiazepine, cannabis and alcohol (Jellinek Laboratory, The Netherlands). Participants were excluded if they reported (1) major medical, neurological or neuropsychiatric illnesses that might affect cognitive function, (2) current use of psychotropic medication, (3)