

Protein kinase B (*AKT1*) genotype mediates sensitivity to cannabis-induced impairments in psychomotor control

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Background. What determines inter-individual variability to impairments in behavioural control that may underlie road-traffic accidents, and impulsive and violent behaviours occurring under the influence of cannabis, the most widely used illicit drug worldwide?

Method. Employing a double-blind, repeated-measures design, we investigated the genetic and neural basis of variable sensitivity to cannabis-induced behavioural dyscontrol in healthy occasional cannabis users. Acute oral challenge with placebo or Δ^9 -tetrahydrocannabinol (THC), the main psychoactive ingredient in cannabis, was combined with functional magnetic resonance imaging, while participants performed a response inhibition task that involved inhibiting a pre-potent motor response. They were genotyped for rs1130233 single nucleotide polymorphisms (SNPs) of the protein kinase B (*AKT1*) gene.

Results. Errors of inhibition were significantly ($p=0.008$) increased following administration of THC in carriers of the A allele, but not in G allele homozygotes of the *AKT1* rs1130233 SNP. The A allele carriers also displayed attenuation of left inferior frontal response with THC evident in the sample as a whole, while there was a modest enhancement of inferior frontal activation in the G homozygotes. There was a direct relationship ($r=-0.327$, $p=0.045$) between the behavioural effect of THC and its physiological effect in the inferior frontal gyrus, where *AKT1* genotype modulated the effect of THC.

Conclusions. These results require independent replication and show that differing vulnerability to acute psychomotor impairments induced by cannabis depends on variation in a gene that influences dopamine function, and is mediated through modulation of the effect of cannabis on the inferior frontal cortex, that is rich in dopaminergic innervation and critical for psychomotor control.

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Introduction

Cannabis is one of the most widely used illicit drugs in the world (United Nations Office on Drugs and Crime, 2010) with evidence of increasing use (Compton *et al.* 2004; Hall & Degenhardt, 2007), accompanied by use

of more potent forms in Europe (Potter *et al.* 2008; European Monitoring Centre for Drugs and Drug Addiction, 2010) and the USA (National Centre for Natural Products Research, 2009). Cannabis use can cause impairments in motor control and impulsive behaviour (Hall & Solowij, 1998; Lane *et al.* 2005; Ramaekers *et al.* 2006, 2009; Weinstein *et al.* 2008a,b; Hall & Degenhardt, 2009), leading to road-traffic accidents (Ramaekers *et al.* 2004; Hall & Degenhardt, 2009) and risk-taking and violent behaviour (Friedman *et al.* 2001; Kingree & Betz, 2003; Lane *et al.* 2005). The inability to exercise restraint and inhibit inappropriate behaviour is a key aspect of impulsivity and is

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characteristic of many neuropsychiatric disorders associated with impulsive behaviours (Chamberlain & Sahakian, 2007). Performance in neuropsychological tests such as response inhibition paradigms and measures of associated neural activity provide an objective measure of deficits in inhibitory control that may underlie impulsivity (Chamberlain & Sahakian, 2007) and impairments in complex motor control functions such as during driving (Fillmore *et al.* 2008). Thus, impairments in psychomotor control and impulsive behaviours under the influence of cannabis may reflect the effect of Δ^9 -tetrahydrocannabinol (THC) (Ramaekers *et al.* 2004), its main psychoactive constituent, on brain areas responsible for response inhibition (Lane *et al.* 2005; Ramaekers *et al.* 2006), such as the inferior frontal gyrus (Rubia *et al.* 2007; Borgwardt *et al.* 2008). However, there is a great deal of variability in the sensitivity of healthy individuals to these effects of cannabis (McDonald *et al.* 2003; Ramaekers *et al.* 2006). It is likely that this may have a genetic basis. However, which particular genes are involved is unclear. In knockout mice, deficiency of protein kinase B (AKT1) has been associated with dendritic ultrastructural abnormalities of pyramidal neurons and alterations in the expression of genes that control synaptic function, neuronal development and myelination in the prefrontal cortex as well as subtle alterations in the performance of prefrontally mediated cognitive tasks such as working memory (Lai *et al.* 2006). Administration of THC has been shown to cause dopamine release in the lateral prefrontal cortex (Stokes *et al.* 2010) and other brain regions in human subjects (Stokes *et al.* 2010) and animals (for a review, see Bhattacharyya *et al.* 2009a). THC has also been shown to activate AKT1 (Ozaita *et al.* 2007), which is an integral component of the dopamine signalling cascade (Beaulieu *et al.* 2007). Studies *in vitro* (Gómez del Pulgar *et al.* 2000; Sánchez *et al.* 2003) have also confirmed that THC and other cannabinoids activate AKT1 and furthermore show that this is probably mediated through their effect on the CB₁ cannabinoid receptor, the main central molecular target of THC (Pertwee, 2008). Consistent with this, variation in the gene coding for the protein kinase AKT1 has been shown to moderate the effects of cannabis on psychosis (van Winkel *et al.* 2011a; Bhattacharyya *et al.* 2012a; Di Forti *et al.* 2012) and on prefrontally mediated cognitive function in those with psychotic disorder (van Winkel *et al.* 2011b) in epidemiological samples. Of the two single nucleotide polymorphisms (SNPs) of AKT1 previously shown to moderate the risk of psychosis associated with cannabis use (van Winkel *et al.* 2011a; Di Forti *et al.* 2012), variation at the rs1130233 SNP has also been associated with a greater risk of short-term psychotomimetic effects of cannabis (van

Winkel *et al.* 2011a). Independent experimental evidence from our group suggesting that variation at the same locus also moderates sensitivity to the symptomatic and neural effects of cannabis (Bhattacharyya *et al.* 2012a) is also consistent with this. For the purposes of the present study, we chose to examine the rs1130233 SNP and not the rs2494732 SNP of AKT1 that was found by previous studies as moderating both the short- as well as the long-term risks of psychosis and cognitive alterations associated with cannabis use (van Winkel *et al.* 2011a,b; Di Forti *et al.* 2012) for a number of reasons. First, the rs1130233 locus of AKT1 is a synonymous coding variant and has been linked to differential expression of the AKT1 protein (Harris *et al.* 2005; Tan *et al.* 2008; Giovannetti *et al.* 2010; Blasi *et al.* 2011) and alterations in the structure and physiology of the prefrontal cortex and prefrontally mediated executive cognition (Tan *et al.* 2008; Pietilainen *et al.* 2009; Blasi *et al.* 2011). On the other hand, the functional consequence of the intronic rs2494732 SNP on protein levels is unclear, and, to the best of our knowledge, variation at this locus has not been linked to any neural activation phenotype such as the one that we investigate here. Furthermore, in our previous study the rs1130233 SNP has been shown to moderate the effects of THC on neural activation during a different cognitive task (verbal memory) that engages the lateral prefrontal cortex and in particular the inferior frontal gyrus (Bhattacharyya *et al.* 2012a). Hence, consistent with a hypothesis-driven candidate-gene approach (Hariri & Weinberger, 2003), we have chosen to focus on the rs1130233 SNP rather than rs2494732, despite emerging evidence linking the latter to the effects of cannabis and psychosis (van Winkel *et al.* 2011a; Di Forti *et al.* 2012). We tested the hypothesis that variation at the AKT1 rs1130233 locus would modulate the acute effects of THC on response inhibition, a prefrontally mediated cognitive process affected by THC, and that this would reflect its influence on the function of the inferior frontal cortex, the principal neural substrate for inhibitory control (Rubia *et al.* 2007).

Method

This study was conducted in accordance with the Declaration of Helsinki after obtaining ethical approval from the local research ethics committee. All participants gave written, informed consent. We employed an established, repeated-measures, placebo-controlled, within-subject, crossover design (Borgwardt *et al.* 2008; Bhattacharyya *et al.* 2012a,b), to examine the acute effects of oral THC on task performance and regional brain activation [blood oxygen level-dependent (BOLD) haemodynamic response] during a well-validated

(Rubia *et al.* 2007; Borgwardt *et al.* 2008) response inhibition task that involves inhibiting a pre-potent motor response and normally engages the inferior frontal cortex (Rubia *et al.* 2007).

Subjects

A total of 36 right-handed, English-speaking, healthy male volunteers [mean age of 25.97 (S.D.=5.58) years and National Adult Reading Test (NART; Nelson, 1982) intelligence quotient (IQ) of 97.7 (S.D.=6)], without a personal or family history of psychiatric illness in first-degree relatives completed the study. Illicit substance use including cannabis use was assessed using the Addiction Severity Index and abuse was defined as 'moderate use of small quantities regularly or large amounts occasionally' (McLellan *et al.* 1980). All subjects completed all of the components of the study and had used cannabis at least once but less than 25 times in their lifetime. None of them used more than 21 units/week of alcohol or other illicit drugs on a regular basis (for information regarding usage of cannabis and other illicit drugs, refer to online Supplementary Table S1). None of them had used cannabis or other illicit drugs for at least 1 month before entering the study and were asked to abstain from all recreational drugs for the duration of the study. All except three (one Chinese and two Sri-Lankan) of the volunteers were of self-reported Caucasian (European) ancestry.

Design

A double-blind, crossover design was used to compare the effects of 10 mg THC, orally administered (approximately 99.6% pure; THC-Pharm, Germany), with matched placebo capsules. Participants were tested on two separate occasions spaced at least 1 month apart. The order of drug administration was pseudo-randomized across subjects, so that an equal number of subjects received any of the drugs during the first or second session. On the day of each session, subjects were required to have a light standardized breakfast after an overnight fast and advised to have at least 6 h sleep the previous night. As smoking nicotine could potentially affect the neural effects of THC and as nine participants in our sample had a lifetime history of smoking nicotine, we asked them to refrain from smoking for 4 h. Of the two participants who had a lifetime use of >10 cigarettes/day in our sample, none was dependent on nicotine and only one smoked at that level around the time of participation in the present study. The other person had stopped smoking 3 years prior to the study. Furthermore, previous research has shown that, while nicotine withdrawal can begin in smokers on the morning after the last smoke

(Schneider & Jarvik, 1984), it does not reach peak intensity until the evening of the second day of abstinence, long after the completion of scanning in the present study. We also reasoned that if a given subject had experienced the effects of nicotine or its withdrawal during the experimental sessions, this would have occurred in conjunction with each drug condition, thus tending to cancel out any effect when these were contrasted with each other. They were also asked to refrain from caffeine for 12 h and alcohol for 24 h.

Subjects were tested for opiates, cocaine, amphetamines, benzodiazepines and THC in urine before each session, using immunometric assay kits. No subjects tested positive for the presence of these substances. Venous blood samples (using an indwelling intravenous catheter inserted into a subcutaneous vein in the forearm of the non-dominant arm) were obtained immediately before, and at 1, 2 and 3 h after drug administration. Blood levels of THC were 2.75 (S.D.=5.83) ng/ml and 4.56 (S.D.=5.20) ng/ml at 1 and 2 h, respectively.

Subjects were scanned using an magnetic resonance imaging (MRI) scanner 1 h following drug administration in a session lasting a maximum of 60 min. Inside the scanner, subjects performed the response inhibition task (described in detail below). Images were acquired between 1 and 2 h after administration of the drug, as our previous work had indicated that a single oral dose produced sustained blood levels over this period (Bhattacharyya *et al.* 2009b) and as the neural and behavioural effects of THC during a response inhibition task (Borgwardt *et al.* 2008) as well as its peak symptomatic effects were also observed within this period (Bhattacharyya *et al.* 2010). Except for the period when MR scanning was performed, subjects remained seated in a quiet room throughout the session.

Response inhibition paradigm (Go/No-Go task)

This paradigm involved a rapid, mixed trial, event-related functional MRI design, with jittered inter-stimulus intervals (ISIs) incorporating random event presentation to optimize statistical efficiency (Dale, 1999). This is a well-validated paradigm requiring either the execution or the inhibition of a motor response depending on the visual presentation of stimuli (Rubia *et al.* 2005a, 2006; Borgwardt *et al.* 2008). The basic 'Go' task is a choice reaction-time paradigm: arrows pointing to either the left or right appeared on the screen for 500 ms with a mean ISI of 1800 ms (range 1600–2000 ms). On 'Go' trials, subjects were instructed to press the left or the right response button according to the direction of the arrow. Infrequently (on 11% of the trials), arrows pointing upward

Table 1. Sociodemographic variables by genotype group

Genotype	Age, years	<i>p</i>	NART IQ	<i>p</i>	Education duration, years	<i>p</i>
<i>AKT1</i> rs1130233						
<i>AKT1</i> (G/G) (<i>n</i> =19) ^a	26.5 (5.8)	N.S.	98.8 (6.9)	N.S.	17.5 (3.2)	N.S.
<i>AKT1</i> (G/A) (<i>n</i> =9)	26.3 (4.7)	N.S.	97.9 (5.3)	N.S.	16.6 (3.3)	N.S.
<i>AKT1</i> (A/A) (<i>n</i> =7) ^b	23.8 (6.6)	N.S.	95.5 (8.0)	N.S.	17.0 (7.4)	N.S.

Data are given as mean (standard deviation).

NART, National Adult Reading Test; IQ, intelligence quotient; AKT1, protein kinase B; N.S., non-significant.

^a Of these, 18 with self-reported white European ethnicity.

^b Of these, five with self-reported white European ethnicity.

appeared. On these 'No-Go' trials, participants were required to inhibit their motor response (and not press any buttons). On another 11% of the trials, arrows pointing to the left or right at a 23° angle were presented. Subjects were told to respond to these the same as for a 'Go' prompt (even though they pointed obliquely). These 'Oddball' stimuli were used to control for novelty effects associated with the low frequency and different orientation of the 'No-Go' relative to the 'Go' trials. In total, there were 24 'No-Go' stimuli, 160 'Go' stimuli and 24 'Oddball' trials and the task duration was 6 min 14 s. Throughout image acquisition, the accuracy and speed of the subjects' button press responses were recorded. Usable performance data were not available from four subjects for the placebo and three subjects for the THC conditions. Subjects practised the entire task once before scanning to ensure familiarity with task demand and to ensure optimal performance.

Image acquisition

All images were acquired on a 1.5 T Signa system (GE Healthcare, USA) at the Maudsley Hospital, London. T2*-weighted images were acquired with echo time (TE) of 40 ms, flip angle 90° in 16 axial planes (7 mm thick), parallel to the anterior commissure–posterior commissure line, on the 1.5 T Signa system.

The Go/No-Go task was studied using a repetition time (TR) of 1800 ms and TE of 40 ms. To facilitate anatomical localization of activation, a high-resolution inversion recovery image dataset was also acquired, with 3 mm contiguous slices and an in-plane resolution of 3 mm [TR=16000 ms, inversion time (TI)=180 ms, TE=80 ms].

Genotyping

Genomic DNA was extracted following standard methods (Freeman et al. 2003). Genotyping of the

AKT1 G>A rs1130233 (Tan et al. 2008) SNP was performed under contract by KBioscience (UK; <http://www.kbioscience.co.uk/>) blind to the results of the THC challenge experiments and was successful in 35 subjects corresponding to a call rate of 97%. The genotype frequencies and the sociodemographic details of the volunteers who completed the study are shown in Table 1. There was no significant difference between the genotype groups with regard to age, NART IQ and number of years of education. Genotype data were tested for deviation from Hardy–Weinberg equilibrium (HWE). Genotype frequencies for *AKT1* at rs1130233 were in HWE ($\chi^2=2.14$, $p>0.05$) in the ethnically stratified sample. In the examination of the modulatory effects of variation at rs1130233 on the various behavioural and imaging parameters, a dominance genetic model contrasted G homozygotes (*n*=19) against the A carriers (*n*=16) (i.e. GG *v.* AG and AA). The A dominant genetic model was favoured, as previous biological work suggests that the A carriers are more likely to show inefficient neural processing. As well as this, recoding for this dominance genetic model also ensured that the genotype groups remained adequately powered throughout these analyses. The A allele has been previously linked with reduced *AKT1* expression (Harris et al. 2005; Tan et al. 2008; Giovannetti et al. 2010; Blasi et al. 2011) and inefficient neural processing phenotype (Tan et al. 2012).

Genetic ancestry

We used a panel of ancestry informative genetic markers to accurately estimate genetic ancestry. All the 35 subjects for whom genotyping was successful were mapped into a three-dimensional ancestral axis, using a genetically validated and published cohort of Black Africans, European Caucasians and Asians (Di Forti et al. 2012) as the genetic reference. This reference set (*n*=215) was used to estimate genetic ancestry. This was done by genotyping a panel of

60 ancestry-informative markers. These markers had a minimum difference in allele frequency of 0.3, between YRI (Yoruba in Ibadan, Nigeria), CEU [Centre d'Etude du Polymorphisme Humain (CEPH) – Utah Residents with Northern and Western European Ancestry] and HCB (Han Chinese in Beijing, China) HapMap populations. The make-up of this marker panel is available upon request. Markers were genotyped using iPLEX technology developed for the MassArray platform (Sequenom Inc., USA). Ancestry scores were derived using the program STRUCTURE which implements a Markov Chain Monte Carlo (MCMC)-based clustering algorithm. The quantitative scores that resulted were used to correct for underlying population substructure that typically hinders the discovery of true association signals. Correcting for underlying population substructure using the ancestry scores, derived as above, did not change the main results of the present study in terms of the effects of variation at rs1130233 of the *AKT1* gene on the effects of THC on task performance as well as neural activity during response inhibition.

Statistical analysis

Behavioural data

Analyses of behavioural data (during the response inhibition task) were performed in SPSS version 21 (IBM, USA). We compared the frequency of inhibition errors between the two drug conditions (THC *v.* placebo) using the χ^2 test. Repeated-measures analysis of variance (ANOVA) was employed to examine the effect of drug conditions (THC *v.* placebo) on reaction time. Association of variation at rs1130233 with the effect of THC on response inhibition errors was tested using logistic regression and effect on reaction time was tested using repeated-measures ANOVA. The overall α level for each hypothesis was fixed at 0.05.

Image analysis

Data from the functional MRI task were analysed using XBAMv4 (<http://www.brainmap.uk/>), employing a non-parametric approach that we have previously used for examining the effects of THC during the same cognitive paradigm (Borgwardt *et al.* 2008). Detailed description of image analysis including pre-processing and control of type 1 error in imaging data is available as online Supplementary material.

For the Go/No-Go task we first contrasted the 'No-Go' and 'Oddball' trials against the 'Go' trials for each drug treatment, to control for activation related to the processing of visually presented arrows on a screen. Brain activation during the successfully performed 'Oddball' trials, which controlled for novelty effects, was then subtracted from brain activation

during the successful 'No-Go' trials ('No-Go' minus 'Oddball') for each drug condition (THC or placebo), to derive brain activation related to response inhibition. Finally, we employed non-parametric repeated-measures ANOVA and a whole-brain analysis approach to identify brain regions that were activated by THC relative to the placebo condition by contrasting the brain activation maps for response inhibition for the two drug conditions. Employing a whole-brain analysis approach, we then examined the main effect of *AKT1* rs1130233 genotype (GG *v.* A carriers) during the task under the placebo condition and the interaction between genotype and drug condition (THC *v.* placebo) for the response inhibition condition.

The relationship between the effects of THC on task performance and its effects on activation (BOLD response) were examined by correlating measures of activation with the change in task performance under the influence of THC, obtained by subtracting task performance under the placebo condition from that under the THC condition. We tested this relationship only in the inferior frontal gyrus, which plays a critical role in inhibitory control, as we had previously predicted that the neural substrate for the effects of THC during response inhibition and its genetic moderation by *AKT1* was going to be the inferior frontal gyrus.

Results

Task performance: effects of THC and genetic modulation

Administration of THC was associated with a significant (Pearson $\chi^2=5.62$, $p=0.018$) increase in the frequency of errors of inhibition relative to the placebo condition (THC: 6.3%; placebo: 3.7%). The effect of THC on response inhibition accuracy was significantly (likelihood ratio test $\chi^2=7.76$, $p=0.02$) influenced by *AKT1* rs1130233 genotype: the frequency of inhibition ('No-Go') errors in A allele carriers was significantly greater (Pearson $\chi^2=7.01$, $p=0.008$) under the influence of THC (7.5%) than placebo (3.1%), while in G homozygotes administration of THC was associated with only a non-significant (Pearson $\chi^2=0.61$, $p=0.4$) increase in errors compared with placebo (5.6% *v.* 4.4%; Fig. 1a). The modulatory effect of genotype on the effect of THC on response inhibition accuracy persisted after correcting for population substructure using ancestry scores derived as above as well as on restricting the analysis to the self-reported Caucasian (European) sample (see online Supplementary Results). This was associated with a significant main effect of drug ($F_{1,9522}=55.13$, $p<0.001$), and an interaction between the effects of the drug and genotype ($F_{1,9522}=14.76$, $p<0.001$) on reaction time (Fig. 1b).

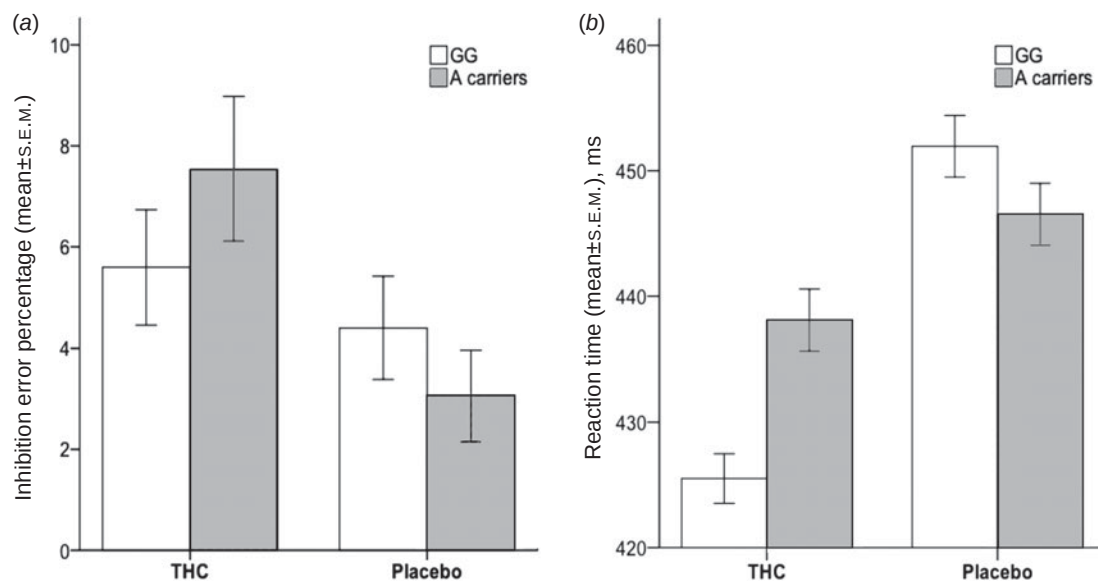


Fig. 1. (a) Modulation of the effects of Δ^9 -tetrahydrocannabinol (THC) on errors of inhibition by the protein kinase B (*AKT1*) rs1130233 genotype [G homozygotes (GG) *v.* carriers of the A allele (A carriers)]. (b) Modulation of the effects of THC on reaction time by the *AKT1* rs1130233 genotype [G homozygotes (GG) *v.* carriers of the A allele (A carriers)].

Thus, while all participants responded faster under the influence of THC than placebo, the speeding of responses was even more marked in G homozygotes than in A allele carriers.

Regional brain activation

Response inhibition task network

Under the placebo condition, response inhibition was associated with activation in the inferior frontal gyrus and adjacent insula on the left side, the middle frontal gyrus extending to the premotor cortex, precentral gyrus and medial frontal gyrus on the right side, extending further to the anterior cingulate gyrus bilaterally as well as the posterior cingulate cortex, precuneus and cuneus (Fig. 2a; online Supplementary Table S2).

Effects of THC on the response inhibition network

During the response inhibition condition, administration of THC was associated with an attenuation of activation in the left inferior frontal gyrus and the adjacent insula, as well as in the left precuneus, relative to the placebo condition. Conversely, there was an augmentation of engagement of the right hippocampus and caudate nucleus under the influence of THC (Fig. 2b; online Supplementary Table S3).

Genetic modulation of the effects of THC on response inhibition

The physiological effects of THC in the inferior and middle frontal gyrus bilaterally, insula, anterior

cingulate/medial prefrontal cortex, pre- and postcentral gyri, medial temporal cortex, lingual gyrus, striatum and cerebellum (Table 2) were significantly modulated by variation at *AKT1* rs1130233. Carriers of the A allele displayed attenuation of the left inferior frontal response with THC evident in the sample as a whole, but in G homozygotes there was a modest enhancement of activation in this region (Fig. 2c, d; Table 2). The modulatory effect of genotype on the effects of THC on the left inferior frontal response persisted after correcting for population substructure using ancestry scores (as a covariate of no interest) as well as on restricting the analysis to the self-reported Caucasian sample. In this part of the inferior frontal gyrus, where *AKT1* genotype modulated the effect of THC, there was a direct relationship ($r = -0.327$, $p = 0.045$) between the physiological effect of THC and its behavioural effect, such that the more THC attenuated inferior frontal activation the greater was the frequency of response inhibition errors.

Discussion

The main finding of the present study is that carriers of the A allele at rs1130233 of *AKT1* have a significantly greater risk of impairment in the control of pre-potent responses under the influence of THC, the principal psychoactive ingredient of cannabis. In carriers of the A allele, which is associated with reduced *AKT1* expression (Harris et al. 2005; Tan et al. 2008; Giovannetti et al. 2010; Blasi et al. 2011), impaired prefrontal executive cognition (Tan et al. 2008;

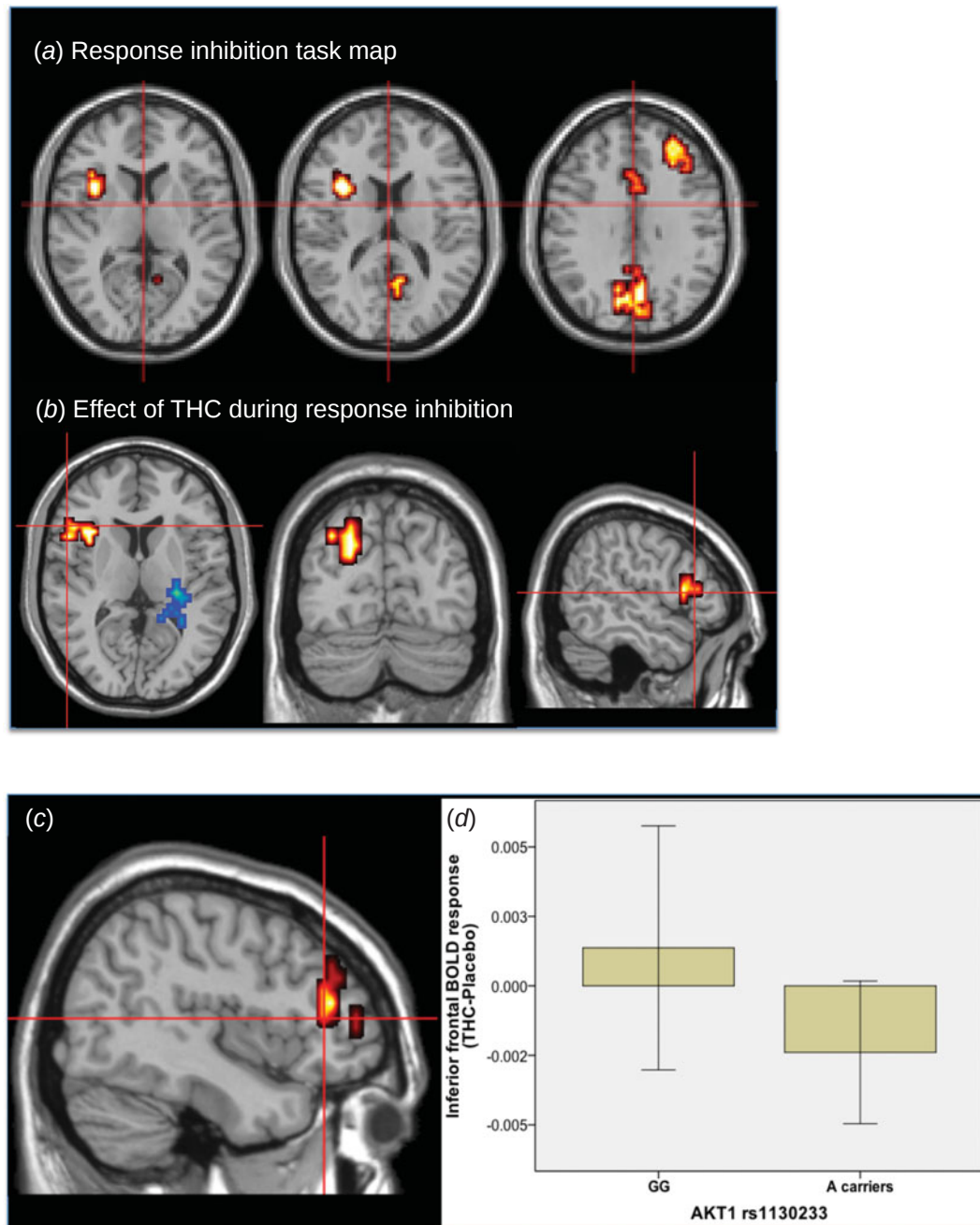


Fig. 2. (a) Regions of the brain engaged by response inhibition under the placebo condition. The left side of the brain is shown on the left side of the image. (b) Effect of Δ^9 -tetrahydrocannabinol (THC) on brain activation during the response inhibition condition in the left inferior frontal gyrus (shown in red and with cross hairs in the transverse and sagittal views), right hippocampus (shown in blue in the transverse view) and precuneus (shown in red in the coronal view). Clusters of activation in red indicate that the contrast shows THC < placebo and those in blue indicate THC > placebo. The left side of the brain is shown on the left side of the image in the transverse and coronal sections. (c) Modulation of the effects of THC on blood oxygen level-dependent (BOLD) response by protein kinase B (AKT1) rs1130233 in the left inferior frontal gyrus (cross hairs: $-43, 37, 9$; $p < 0.0035$ corrected for < 1 false positive). (d) Magnitude of BOLD response (indexed by mean sum of squares ratio, with standard error of the mean, in arbitrary units on the y-axis).

Blasi *et al.* 2011) and inefficient neural processing (Tan *et al.* 2012), administration of THC was associated with an over two-fold increase in the frequency of

impairments in inhibitory control and a concomitant attenuation of the engagement of the inferior frontal gyrus, the principal neural substrate for inhibitory

Table 2. Modulation of the effects of THC by *AKT1* rs1130233 during the response inhibition condition of the Go/No-Go task ($p < 0.0035$; corrected for <one false-positive cluster)

Size	x	y	z	Side	Cerebral region
10	-43	37	9	L	Inferior frontal gyrus
14	25	30	-7	R	
20	-32	52	-2	L	Middle frontal gyrus
7	22	37	-13	R	
14	29	19	4	R	Insula
7	25	-19	37	R	Precentral gyrus
20	22	-26	48	R	Postcentral gyrus
36	-22	-4	-2	L	Striatum
	-22	-4	-7	L	
	-22	-4	4	L	
	-25	4	9	L	
9	29	-4	20	R	
28	14	33	-7	R	Anterior cingulate/medial prefrontal cortex
10	-22	-7	-13	L	Hippocampus/amygdala
8	11	-67	-2	R	Lingual gyrus
24	11	-67	-7	R	Cerebellum

THC, Δ^9 -tetrahydrocannabinol; AKT1, protein kinase B; x, y, z, Talairach region x, y and z planes; L, left; R, right.

control. Furthermore, there was a direct relationship between the effects of THC on behaviour (psychomotor control) and the physiological response of its neural substrate, such that the more THC attenuated inferior frontal engagement, the greater was the risk of errors of inhibition. These results are consistent with evidence that the inferior frontal gyrus plays a critical role in inhibitory control (Rubia *et al.* 2001, 2005a, 2007), with greater prefrontal activation being related to better inhibitory control (Aron *et al.* 2007; Rubia *et al.* 2007). In previous neuroimaging studies that have related inferior frontal activation to inhibitory control, this has been generally found to be right lateralized (Garavan *et al.* 1999; Aron *et al.* 2007; Eagle *et al.* 2008), unlike in the present study. However, numerous other studies have also reported bilateral inferior frontal activation in the context of various response inhibition paradigms including the Go/No-Go task, with a predominantly bilateral pattern of activation reported by studies employing the Go/No-Go task (Kawashima *et al.* 1996; Casey *et al.* 1997; Krams *et al.* 1998; Menon *et al.* 2001; Rubia *et al.* 2001, 2005b; Durston *et al.* 2002; Fassbender *et al.* 2004; Li *et al.* 2006; Eagle *et al.* 2008), suggesting that the left-lateralized pattern of effect of THC and its genetic modulation may reflect the specific variant of response inhibition paradigm employed in the present study. Impairment in the inhibition of motor responses under the influence of THC has been reported previously in some (McDonald *et al.* 2003; Ramaekers *et al.* 2006) but not all studies (McDonald *et al.* 2003). Results of the present study

suggest that genetic variation in the individual sensitivity to the effects of THC may contribute to such inconsistencies. Administration of THC was also associated with faster responding to task stimuli, consistent with previous evidence (Skosnik *et al.* 2001; Curran *et al.* 2002), but this effect was more marked in G homozygotes rather than A allele carriers of *AKT1*. Thus, the greater sensitivity of *AKT1* A allele carriers to inhibitory dyscontrol under the influence of THC was not just a result of faster responding under its influence, but a specific impairment in inhibiting a prepotent response. This may appear counterintuitive and seem to contradict the main results of this study that suggest a greater sensitivity of the A allele carriers to the neural and behavioural effects of THC. However, this may also suggest that greater sensitivity of the A allele carriers may selectively affect behavioural control as measured by errors of inhibition and does not extend to other aspects of the broader construct of impulsivity that are tapped by the Go/No-Go task, such as speeding up while responding. Homozygotes of an *AKT1* risk allele at a locus (rs2494732) likely to be in strong linkage disequilibrium (Di Forti *et al.* 2012) with the locus (rs1130233) tested here have been previously reported to be sensitive to the adverse effects of cannabis use on performance accuracy during a selective attention task with concomitant slowing down of responding (van Winkel *et al.* 2011b). Results presented in our sample may also reflect a compensatory slowing down in the A allele carriers under the influence of cannabis, which, however, failed to

compensate for their impairment in inhibitory control. Compensatory behaviour while driving under the influence of cannabis under experimental driving simulation conditions and in real life has been reported (Ramaekers *et al.* 2004).

Collectively, these results indicate that individuals carrying the A allele of *AKT1* rs1130233 may have increased sensitivity to impairments in psychomotor control caused by cannabis, which is in turn mediated through an effect of THC on the inferior frontal cortex, the principal neural substrate for inhibitory control (Rubia *et al.* 2007). Impairments in a similar inhibitory control paradigm are strongly correlated with errors during a simulated driving task (Fillmore *et al.* 2008), and cannabis is one of the commonest psychoactive substances related to road-traffic accidents (Ramaekers *et al.* 2004). Doses of THC comparable with that administered in the present study have been shown to cause more severe impairments in actual driving performance under experimental conditions than while driving with blood alcohol concentrations over the legally accepted limit in many European countries (Ramaekers *et al.* 2000). Our findings suggest that the marked variability in susceptibility to these adverse effects of cannabis use has a genetic basis, and is specifically related to a gene that influences dopamine signalling in the brain. However, it is important to note that driving is a complex operation that involves different cognitive processes including strategic processes such as planning, tactical processes such as manoeuvring as well as operational processes, of which, exerting psychomotor control is but only one aspect (Salvucci, 2006). Hence, while the results of the present study may help explain why certain individuals may be sensitive to the adverse effects of cannabis use on the psychomotor control aspects of driving, road-traffic accidents are likely to be related to impairments in any of the other processes referred to earlier, operating either alone or in combination.

Impairments in the control of behaviour are also well known under the influence of cannabis (Hall & Solowij, 1998), leading to violent (Friedman *et al.* 1996, 2001; Resnick *et al.* 1997; White *et al.* 1999) and risky or impulsive behaviour (Watts & Wright, 1990; Fergusson & Horwood, 1997; Duncan *et al.* 1999; Kingree & Betz, 2003). However, inconsistent findings from epidemiological (Macleod *et al.* 2004) and experimental (McDonald *et al.* 2003; Lane *et al.* 2005; Ramaekers *et al.* 2006) studies suggest that there is a great deal of variability in the effects of cannabis and that the function of behavioural control in humans is not affected by cannabis to the same extent in all individuals. The results of this study suggest that this variation in individual sensitivity to cannabis may coincide with variation at the rs1130233 locus in the

AKT1 gene. It is important to emphasize that the effects attributed to variation at the rs1130233 locus should be seen in the context of a broader polygenic framework within which variation at rs1130233 may contribute towards a small proportion of the total variance attributable to genetic factors. However, our results are consistent with the modulatory effects of THC on *AKT1* phosphorylation (Gómez del Pulgar *et al.* 2000; Sánchez *et al.* 2003; Ozaita *et al.* 2007) and evidence that vulnerability to the psychotogenic effects of cannabis are mediated by the same genetic variant (van Winkel *et al.* 2011a; Bhattacharyya *et al.* 2012a). They are also consistent with recent evidence that a related variation in the *AKT1* gene moderates the longer-term effects of cannabis on measures of sustained attention, a process that relies on prefrontal function (van Winkel *et al.* 2011b). Moderation of the effects of THC in the prefrontal cortex by a genetic variant that modulates central dopaminergic neurotransmission is also consistent with the rich dopaminergic input to this region (Seamans & Yang, 2004) and evidence that THC affects central dopaminergic neurotransmission in animals (Chen *et al.* 1990a,b; French *et al.* 1997; Tanda *et al.* 1997) and human subjects (Voruganti *et al.* 2001; Bossong *et al.* 2009; Stokes *et al.* 2010). Modulation of change in cognitive function associated with schizophrenia, a disorder characterized by dopamine dysregulation (Howes & Kapur, 2009), by an interaction between *AKT1* rs1130233 SNP and other pharmacological activators of *AKT1* has also been reported (Tan *et al.* 2012).

To our knowledge, this is the first study to demonstrate that genetic variation moderates the variability in sensitivity to the acute impairments in psychomotor control induced by THC and describes how this effect is mediated in the brain. These effects may underlie the effects of cannabis on impaired motor control, and impulsive, violent and risky behaviour. One of the principal strengths of this study is the use of an experimental design in occasional users of cannabis that allowed examination of the genetic moderation of the effect of the drug on behaviour and its neural substrate without the confounding effects of variation in the dose, composition and duration of cannabis use as well as differences between regular users *versus* non-users. At the population level, these various confounding factors may interact with genetic vulnerability to modulate the effects of cannabis. Nevertheless, the results of the present study may serve as a reference point for future studies examining the genetic and other determinants of vulnerability to the acute and long-term effects of cannabis use at the population level.

However, the results of the present study need to be interpreted in the light of certain caveats. One of the concerns in genetic association studies of this nature

relates to the effects of population stratification, especially because of the heterogeneous ethnic make-up of our sample. In order to address this issue, we have used genetic markers to accurately estimate ancestry. Correcting for underlying population substructure, using the ancestry scores derived as described in the Method section, did not change the main results of the present study in terms of the effects of variation at the rs1130233 locus of the *AKT1* gene on the effects of THC on task performance as well as neural activity during response inhibition. Furthermore, reanalysis of the data after excluding the three participants of self-reported Asian origin did not change the main conclusions of the study regarding genetic modulation of the effects of THC on inferior frontal activation and task performance during the response inhibition condition (data available on request). Another potential area of concern may be related to the size of the present sample, which is modest by the standards of traditional genetic association studies. However, the behavioural and genetic effects reported here are statistically significant and the size of the genetic subgroups examined here are consistent with existing practice in the field and samples employed in previous studies that have examined single-gene effects (Egan *et al.* 2001). Furthermore, we employed a repeated-measures within-subject design and employed a hypothesis-driven approach that involved the choice of a candidate genetic variation that has previously been shown to be functional (altering the expression of a protein that is activated by THC) and linked to prefrontal physiology and the effects of THC, and, as a result, the present study had sufficient power to detect modulatory effects of genetic variation, as is evident from the results presented. Greater sensitivity of the functional MRI signal to experimental perturbations relative to behavioural measures and its greater proximity to the underlying molecular genetic variation also allowed us to detect a robust effect of genetic variation on the functional MRI signal with a much smaller sample size than would be feasible with traditional genetic association studies (Hariri & Weinberger, 2003). Nevertheless, the results presented here need independent replication and extension to larger samples. As we administered pharmacological-grade THC and not cannabis *per se*, it may be argued that the results may not be generalizable to the effects of the cannabis that is available on the street, which has many different ingredients with varying and, sometimes, opposite effects (Bhattacharyya *et al.* 2010). While that is true, THC is the principal psychoactive ingredient in cannabis, responsible for most of its adverse effects on cognition (Adams & Martin, 1996; Hall & Solowij, 1998), including impairments in psychomotor control (McDonald *et al.* 2003; Ramaekers *et al.* 2004, 2006;

Bhattacharyya *et al.* 2010). Hence, to the extent that the effects of cannabis in the street reflect the effects of THC, the results of the present study provide the first experimental evidence that individual sensitivity to impairments in psychomotor control induced by cannabis is mediated by the effect of a genetic variation on brain function. Furthermore, the results of the present study have particular relevance to the effects of cannabis on behaviour at the population level, as there is accumulating evidence that the THC content of cannabis that is available on the street is increasing, making it more potent (Potter *et al.* 2008; National Centre for Natural Products Research, 2009; European Monitoring Centre for Drugs and Drug Addiction, 2010). Another caveat relates to the mode of administration of THC in this study. Smoking is the most common mode of using cannabis, unlike the oral route employed in the present study. This route of administration was employed in the present study because it allows a more sustained dose of THC than the inhaled route and ensured that adequate levels of THC were present throughout the period of the functional MRI scan. While the time-course and severity of the effects of THC or cannabis may vary depending on the route of administration, the effects being faster in onset and more severe in intensity but shorter in duration with the inhalation route (Ohlsson *et al.* 1980), the present study provides robust evidence under controlled experimental conditions of the adverse pharmacological effects of THC on psychomotor control. Functional neuroimaging studies suggest that the effects of cannabis in the brain are comparable between studies that employ various routes of administration (Martin-Santos *et al.* 2010). A further caveat may be related to the high inhibition success rate in the Go/No-Go task across the two placebo and THC conditions, suggesting that the task was not particularly challenging for the study participants. Nevertheless, inhibition success in the present study is comparable with that in previous studies using identical or different versions of the task in healthy individuals (Menon *et al.* 2001) as well as under the influence of THC (Borgwardt *et al.* 2008). In this context, a further limitation that is worth considering relates to the moderate size of the correlation between the behavioural (inhibition errors) and neural (inferior frontal engagement) effects of THC.

It may also be argued that the present study was conducted in occasional cannabis users and hence the results are not generalizable to the vast number of cannabis users who use it on a regular basis. It is true that other factors such as tolerance and sensitization may alter the behavioural and neural response in regular users of cannabis. Nevertheless, the results of the present study are relevant to the adverse effects on

behaviour experienced by regular users when they start experimenting with cannabis as well as the huge majority of people who only use it occasionally and never go on to become regular users. Furthermore, evidence suggests that tolerance may not develop to the impairments in psychomotor control induced by cannabis following regular use (Ramaekers *et al.* 2009).

Conclusion

Together, the results of this study provide preliminary evidence that warrants independent replication in larger samples, suggesting that variation in a gene that codes for a protein which influences central dopaminergic transmission may moderate sensitivity to the acute impairments in psychomotor control induced by THC that may underlie the effects of cannabis on impaired motor control, and impulsive, violent and risky behaviour and that this effect is mediated through an effect on the key neural substrate for inhibitory control.

Supplementary material

For supplementary material accompanying this paper visit <http://dx.doi.org/10.1017/S0033291714000920>.

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Declaration of Interest

None.

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