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GABA Deficits Enhance the Psychotomimetic Effects of Δ^9-THC

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INTRODUCTION

Emerging evidence supports a number of associations between cannabis and psychosis/psychotic disorders, including schizophrenia. Acute exposure to both cannabis and synthetic cannabinoids (CBs) (including Spice and K2) can produce a range of transient psychotomimetic symptoms, cognitive deficits, and psychophysiological abnormalities that bear a striking resemblance to symptoms of schizophrenia (D'Souza *et al*, 2004; Radhakrishnan *et al*, 2014). Furthermore, epidemiologic studies suggest that early and heavy exposure to cannabis confers a higher risk for developing a psychotic disorder such as schizophrenia (Moore *et al*, 2007). However, only a minority of individuals exposed to CBs appear to be vulnerable to CB-related acute or persistent psychosis outcomes.

In individuals with schizophrenia, CBs have been shown to *transiently* exacerbate symptoms (D'Souza *et al*, 2005), trigger relapse, and have negative consequences on the course of the illness (Linszen and van Amelsvoort, 2007). A number of lines of evidence suggest that schizophrenia patients are more vulnerable to some of the effects of CBs. In an experience sampling study, schizophrenia patients were more sensitive to the psychosis-inducing effects of cannabis than controls (Henquet *et al*, 2010). Epidemiologic studies also show that cannabis use is associated with greater negative consequences on the course and expression of schizophrenia (van Os *et al*, 2002). In an experimental study, despite receiving stable doses of antipsychotic medications and being clinically stable, 80% of schizophrenic patients, but only 25% of controls, experienced clinically significant psychosis (>3 points on the Positive and Negative Syndrome Scale (PANSS) positive subscale) with a low dose of delta-9-tetrahydrocannabinol (THC) (D'Souza *et al*, 2005). Finally, individuals who are psychosis prone as determined either psychometrically or by family history are more sensitive to the psychosis-inducing effects of cannabis (Arendt *et al*, 2008; GROUP, 2011). However, the basis of the enhanced vulnerability to the psychosis-inducing effects of CBs in schizophrenia patients is not clear. Several other mechanisms might explain vulnerability to THC effects including polymorphisms of genes for COMT (Henquet *et al*, 2006), AKT1, and DAT1 (Bhattacharyya *et al*, 2012, 2014), and γ-aminobutyric acid (GABA) deficits. Furthermore, it is conceivable that combinations of these factors may coexist and have additive or synergistic effects on increasing vulnerability to THC effects.

GABA deficits have been observed in the dorsolateral prefrontal cortex in schizophrenia (Lewis *et al*, 2005), and furthermore, there is important interplay between the CB and GABA systems (Eggan *et al*, 2010). Indeed, converging lines of evidence, including post-mortem (Lewis *et al*, 2005), genetic (reviewed by Charych *et al*, 2009), and brain imaging studies (Busatto *et al*, 1997; Verhoeff *et al*, 1999) suggest that dysfunction of the GABA system contributes to the pathophysiology of schizophrenia. Although there is strong support for the existence of a GABA deficit in schizophrenia, the proportion of schizophrenia patients with this deficit is not known. The limited data available suggest that only 50% of schizophrenia patients have lower GABA levels compared with the lowest level found in healthy normal controls (Yoon *et al*, 2010).

In several brain regions, particularly the cerebral cortex and hippocampus, CB1 receptors (CB1-Rs) are present on the axon terminals of cholecystokinin (CCK) containing GABA interneurons that target the perisomatic region of pyramidal cells (PCs) (Eggan *et al*, 2010). CB1-Rs are activated by endocannabinoids released postsynaptically by depolarized PCs (Wilson and Nicoll, 2002). The activation of CB1Rs inhibits the release of GABA by CCK-basket cells, leading to a disinhibition of postsynaptic PCs

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(Bartos and Elgueta, 2012; Klausberger *et al*, 2005). Thus, a CB1-R-mediated braking mechanism regulates the timing and release of GABA, and subsequently the overall inhibitory/excitatory balance in cortical networks (Farkas *et al*, 2010). This interplay between GABA and CB1-R systems provides a mechanism that could explain the higher vulnerability to CBs in schizophrenia. For instance, if CB1-R activation occurred in the presence of a pre-existing GABA deficit (as might be the case in schizophrenia), this could lead to further disinhibition and desynchronization of PC activity, leading to perturbations in gating, associative functions, and neurocognition, which could culminate in psychotic symptoms.

This study tested the hypothesis that if, among other mechanisms, GABA deficits contribute to the increased vulnerability of schizophrenia patients to the psychosis-exacerbating effects of CBs, then inducing a GABA deficit in healthy subjects will increase the psychosis-inducing effects of THC. As described below, a GABA deficit was pharmacologically modeled by the administration of the $GABA_A$ inverse agonist, iomazenil.

MATERIALS AND METHODS

In a four test day double-blind, placebo-controlled, randomized and counterbalanced study, healthy volunteers received iomazenil followed by THC, placebo iomazenil followed by THC, iomazenil followed by placebo THC, and placebo iomazenil followed by placebo THC. The study was conducted in the Neurobiological Studies Unit (VA Connecticut Healthcare System (VACHS), West Haven, CT). Subjects were recruited by advertisements and by word of mouth, and were paid for their participation.

Approvals

The study was approved by the institutional review boards of the VACHS and Yale University School of Medicine and was carried out in accordance with the Declaration of Helsinki (1975). The study was carried out under Investigational New Drug applications (51 671 and 75 099). Subjects were informed about the potential for adverse effects of THC, iomazenil, and the combination.

Subjects

After obtaining written informed consent, subjects ($n = 27$) underwent a Structured Clinical Interview for DSM-IV (SCID; First *et al*, 2002) and were carefully screened (Supplementary Text no. 1). Women were excluded because the teratogenicity of iomazenil is not known. A detailed history of cannabis exposure was obtained. Cannabis-naive individuals were excluded to minimize any risk of promoting future cannabis use/abuse. Exclusion criteria included a personal or family history of epilepsy or seizure disorder because of the potential for iomazenil to reduce seizure threshold, a family history of a major Axis 1 disorder, and DSM-IV cannabis or other substance dependence (except nicotine). A general physical and neurological examination, electrocardiogram, and laboratory tests were also conducted. A screening electroencephalogram (EEG) was conducted to exclude subjects with any activity suggestive of seizure disorder. After screening, subjects were instructed to refrain from alcohol, caffeinated beverages, illicit drugs (other than cannabis), or prescription drugs not approved by the research team for 1 week before the study and throughout study participation. Compliance with the alcohol and drug prohibitions was formally assessed at every screening visit and on the morning of each test day (×4), by asking subjects and also testing breath for alcohol, and urine for drugs. Compliance with the caffeine restriction was checked by asking subjects.

Drugs

The preparation, formulation, and storage of THC solution and control are reported elsewhere (D'Souza *et al*, 2004) (Supplementary Text no. 2). THC at a dose of 0.015 mg/kg (1.05 mg in a 70 kg individual) was administered intravenously over 10 min into a rapidly flowing intravenous infusion of normal saline.

Iomazenil (Ro 16-0154) is an iodine analog of the benzodiazepine receptor (BZR)-competitive antagonist flumazenil. Some of its pharmacologic properties are comparable to those of the BZR-competitive antagonist flumazenil (Beer *et al*, 1990). However, unlike flumazenil, which blocks the effects of BZR agonists but lacks intrinsic pharmacological effects (Hunkeler *et al*, 1981), inverse agonists interfere with the function of the BZR in coupling the $GABA_A$ receptor and chloride channel, and have intrinsic pharmacological effects opposite to those of BZR agonists (Tallman and Gallager, 1985). For example, the BZR inverse agonist FG-7142 is anxiogenic in healthy human subjects (Dorow *et al*, 1983; Horowski and Dorrow, 2002). BZR inverse agonist drugs bind to extrasynaptic $GABA_A$ receptors (Liang *et al*, 2004). Iomazenil has high affinity and selectivity for BZRs ($K_d = 0.5$ nM) (Johnson *et al*, 1990). In *in vitro* models, it inhibits the binding of the weak BZR inverse agonist [^{3}H] Ro15-4513 to γ2- and δ-subunit-containing $GABA_A$ receptors with IC_{50} of 20–30 nM (R Olsen, personal communication, 26 January 2007). Iomazenil produces a net deficit in GABA function; in preclinical studies, it has been shown to behave as a BZR-competitive antagonist with inverse agonist effects (Beer *et al*, 1990; Hoffmann-La Roche; Schubiger and Hasler, 1989), and in clinical studies, it has been shown to have anxiogenic effects, and proconvulsant effects at higher doses (Randall, 1995; personal communication). Consistent with a role of GABA deficits in the pathophysiology of psychosis, iomazenil has been shown to increase the psychotomimetic effects of the 5-HT_2 partial agonist 1-(*m*-chlorophenyl)piperazine (*m*-CPP) in healthy subjects (D'Souza *et al*, 2006) and schizophrenia patients are more vulnerable to the propsychotic effects of iomazenil (Ahn *et al*, 2011). Iomazenil was administered intravenously at a dose of 3.7 μg/kg over 10 min.

Blood was sampled repeatedly and assayed for THC and 11-nor-Δ-9-THC-9-COOH (THC-COOH) using gas chromatography mass spectrometry (Shaw *et al*, 1991) to rule out that any hypothesized worsening of effects with the combination of the two drugs was not merely a result of iomazenil increasing THC levels.

Behavioral and Subjective Measures

Psychosis-relevant symptoms were assessed using the PANSS (Kay *et al*, 1989). Perceptual alterations were measured using the Clinician Administered Dissociative Symptoms

Scale (CADSS) (Bremner *et al*, 1998). 'High' and other subjective effects associated with cannabis intoxication were measured using a self-reported visual analog scale (VAS) (0–100). These assessments were administered at baseline (−60 min), +70 min and +240 min timepoints, where timepoint 0 min denotes the beginning of the THC infusion. The baseline rating was to assess the predrug state. Subsequent ratings covered the entire time period between the current and past timepoint, for example, +70 covered the time period from baseline to +70 min. The same research coordinators rated all 4 test days for each subject. Inter-rater reliability sessions were conducted every 1–2 months over the time period that this study was conducted (~4 years) and, for example, intraclass correlation coefficients for the PANSS were consistently >0.85 (Table 1).

General Procedure and Test Days

Test days were separated by 3 days to minimize carryover effects given the half-life of THC. Subjects fasted overnight, reported to the test facility around 0800 hours, and were provided a standard breakfast. Urine toxicology was conducted on the morning of each test day to rule out recent illicit drug use. In-study safety procedures are described elsewhere (D'Souza *et al*, 2004). Behavioral and subjective ratings, vital signs, and blood sampling were repeated several times before and after drug administration, while psychophysiological data were collected only once per test day. A field sobriety test, mental state examination, and exit interview were conducted at the end of each test day. Prospective safety assessments were performed at 1 and 3 months after the last test session and after they had received payment for participation to query their use of cannabis.

EEG Recording

EEG data were collected in an acoustically shielded booth, and recording was carried out with the commercially available Active Two Acquisition System (Biosemi, The Netherlands). A sampling rate of 1024 Hz was used, with online low-pass filter of 256 Hz to prevent aliasing of high frequencies. A 64-channel electrode cap according to the extended 10-20 system was used, along with additional electrodes to record the vertical and horizontal electro-oculogram. All electrodes were referenced during recording to a common-mode signal electrode between POz and PO3 and then subsequently re-referenced to the nose offline.

EEG Task

A three-stimulus auditory oddball task adapted from our previous study was used (D'Souza *et al*, 2012). Briefly, a random series of infrequent (8.33%) 'target' tones (1000 Hz sine wave), frequent (83.33%) 'standard' tones (20, 30, or 40 Hz click trains), and infrequent distractor sounds (8.33%) were presented with a 1250 interstimulus interval in three separate blocks. Distractors included a set of novel everyday natural and manmade sounds (Friedman *et al*, 1993). Target tones and standard click trains were 500 ms in duration, whereas distractor stimuli ranged in duration from 175 to 250 ms. All stimuli were presented binaurally using at an intensity of 80 dB SPL.

Table 1 Schedule of Test Day

Time	Procedure
Screening (~4 weeks before test day)	• Medical and psychiatric history, SCID, cannabis/drug/alcohol use, confirmation of history with collateral • Lifetime marijuana use assessment • Chemistry, hematology, urine toxicology, EKG, vital signs, height, and weight • Baseline safety EEG to rule out risk for seizures

−120	• Confirm adherence to prestudy prohibitions:
	  • Last use of tobacco, cannabis, alcohol, caffeine, other drugs, medications, and supplements
	  • Breathalyzer
	  • Urine toxicology
	• Confirm that subjects have not had any recent psychosocial stressors?
	• Confirm that subject has fasted since midnight?
	• Vital signs: blood pressure and heart rate
	• Standard light breakfast
	• Insert two IV lines: identify arms for iomazenil and THC infusions

−90	• Set up EEG cap • Vital signs: blood pressure and heart rate
−60	• Ratings: PANSS, CADSS, and VAS • Blood sampling for THC/THC-COOH assay
−15	• Vital signs: blood pressure and heart rate
−10	• Intravenous iomazenil 3.7 μg/kg over 10 min
0	• Intravenous THC (0.015 mg/kg) or placebo over 10 min
+5	• Vital signs: blood pressure and heart rate
+10	• Vital signs: blood pressure and heart rate • Blood sampling for THC/THC-COOH assay
+25	• Event-related potentials: P300 • Vital signs: blood pressure and heart rate
+45	• Vital signs: blood pressure and heart rate
+70	• Ratings: PANSS, CADSS, and VAS
+80	• Vital signs: blood pressure and heart rate • Blood sampling for THC/THC-COOH

+240	• Vital signs: blood pressure and heart rate
	• Ratings: PANSS, CADSS, and VAS
	• Blood sampling for THC/THC-COOH assay
	• Safety check list:
	  ○ MMSE
	  ○ Field sobriety test
	  ○ Exit interview
	  ○ Discharge instructions

Abbreviations: SCID, Structured Clinician Interview; EEG, electroencephalography; NPO, nil per oral; PANSS, Positive and Negative Syndrome Scale; CADSS, Clinician Administered Dissociative Symptoms Scale; VAS, Visual Analog Scale; MMSE, Mini Mental State Examination. Safety follow-up: 1 and 3 months after last test day for cannabis use and psychiatric symptoms.

In each block, participants were asked to press a response key to the target stimuli with the index finger of their preferred hand. Each block was comprised of 15 targets, 15 distractor stimuli, and 150 standards (20 Hz standard click trains for Block 1, 30 Hz standard click trains for Block 2, and 40 Hz standard click trains for Block 3). The order of blocks was randomized for each test day. To maximize

event-related potential (ERP) signal-to-noise-ratio, target and distractor stimuli were averaged from all three blocks. Thus, in total, there were 45 targets, 45 distractors, and 450 standards.

EEG Signal Processing

EEG data were first bandpass filtered from 0.1 to 100 Hz (24 dB/oct) and notch filtered at 60 Hz. The recorded EEG was then segmented into epochs consisting of a 100 ms baseline and ending 1000 ms after stimulus onset. Ocular movement correction was applied using Gratton's algorithm (Gratton *et al*, 1983). After baseline correction, any trial with a voltage $> \pm 100\,\mu V$ was excluded from analysis. Finally, the data were low-pass filtered (12 Hz cutoff, 24 dB/oct) and single trials were averaged before peak detection of ERPs.

For target and novel stimuli, the P300b and P300a, respectively, were identified as the largest positive voltage peak between 250 and 400 ms after stimulus onset using an automated algorithm. To assess primary sensory processing and registration, the N100 component to both target and novel stimuli was examined. The N100 was defined as the largest negative voltage peak between 50 and 150 ms after stimulus onset. For statistical analysis, data were used for each component where amplitude was largest as described previously (Pz for P300b; Cz for P300a; Cz for N100) (D'Souza *et al*, 2012). Processing and analysis of distractor stimuli will be reported elsewhere. All EEG processing was performed using commercially available software (Analyzer 2.0; Brain Products GmbH, Gilching, Germany).

Statistical Analysis

Initially, data were examined descriptively using means, SDs, and graphs. Each outcome was assessed visually for normality using histograms and normal probability plots. As THC peak effects were captured 70 min after drug administration, and because at other timepoints there was little difference from the minimum score, each behavioral and subjective outcome was expressed as peak change from baseline using methods described elsewhere (D'Souza *et al*, 2012). The data were analyzed using linear mixed models with drug condition: (1) active iomazenil, placebo THC, (2) active iomazenil, active THC, (3) placebo iomazenil, active THC, and (4) placebo iomazenil, placebo THC as a within-subjects factor. Tukey's *post-hoc* procedure was used to determine significant pair-wise group differences between each of the four conditions. The correlation between repeated measures on an individual was modeled using random-effects and/or structured variance–covariance matrices. The best fitting variance–covariance structure was determined using information criteria. In the above models, potential covariates (eg, frequency of and days since last use of cannabis) were entered into the model in turn but were not significant and dropped for parsimony. The mixed-effects approach is advantageous as it is unaffected by randomly missing data and allows greater flexibility in modeling the correlation structure of repeated-measures data (Gueorguieva and Krystal, 2004). Models similar to above were used to analyze P300a and P300b data. All analyses were conducted using SAS version 9.1 (SAS Institute Inc., Cary, NC) (Table 2).

Table 2 Sample Demographics

	Mean	SD
Age (years)	25.44	7.41
Weight (kg)	80.98	12.19
Height (cm)	178.87	6.96
IQ (NART)	116.85	5.27
Psychosis proneness		
Wisconsin Psychosis Proneness	27.30	10.75
SPQ (Schizotypal Personality Questionnaire)	5.87	5.12
Average number of alcoholic drinks per week	5.8	5.9
Cannabis use		
Age at first use	17.27	2.35
Frequency of use in past 30 days (no. of days of use)	6.22	7.73
Lifetime frequency of use (no. of days of use)	296.75	266.26
	Total *n*	
Handedness (no.)	22	
Right		
Left	4	
Ambidextrous	1	
Cigarette smoker	4	
Yes		
No	23	
No. of subjects who ever tried drugs other than cannabis		
None	7	
Hallucinogens	15	
Stimulants	8	
Opiates	2	
Inhalants	0	

*None of the subjects met the criteria for abuse or dependence of alcohol and illicit drugs of abuse.

RESULTS

Of the 27 male subjects who were enrolled in the study, 21 completed all 4 test days. EEG data was collected in 23 subjects. The demographic characteristics of the sample are listed in Tables 2 and 3.

Plasma Level of THC and THC-COOH

There were no significant effects of iomazenil on plasma levels of THC (drug condition × time, $p = 0.46$) and THC-COOH (drug condition × time, $p = 0.18$) (Supplementary Figure 1).

Behavioral and Subjective Effects

For all behavioral and subjective measures, the main analysis of interest was the contrast between the conditions of

Table 3 Behavioral and Subjective Effects of THC and Iomazenil Mean (SD) Scores on the PANSS, CADSS, and VAS Across Drug Condition and Time, Along With *Post-Hoc* Contrasts

Condition	Timepoint	*N*	Mean	SD	Significant *post-hoc* contrasts
PANSS total score					
IOM − /THC −	− 60	23	28.83	0.78	IOM+/THC+ *vs* IOM − /THC+ ($p = 0.038$)
	70	23	33.26	6.33	
	240	23	28.17	6.64	IOM+/THC+ *vs* IOM+/THC − ($p = 0.003$)
IOM+/THC −	− 60	20	28.65	0.59	
	70	20	38.1	8.84	IOM+/THC+ *vs* IOM − /THC − ($p < 0.0001$)
	240	20	28.5	0.69	
IOM − /THC+	− 60	23	28.83	0.78	IOM − /THC+ *vs* IOM − /THC − ($p = 0.005$)
	70	23	40.83	7.69	
	240	23	28.96	1.3	
IOM+/THC+	− 60	23	28.7	0.63	
	70	21	46.19	9.79	
	240	21	29.19	1.89	
CADSS patient-rated					
IOM − /THC −	− 60	23	0.17	0.49	
	70	23	1.26	3.09	IOM+/THC+ *vs* IOM+/THC − ($p = 0.029$)
	240	23	0.04	0.21	
IOM+/THC −	− 60	20	0	0	IOM+/THC+ *vs* IOM − /THC − ($p = 0.001$)
	70	20	4.65	7.11	
	240	20	0.15	0.49	IOM − /THC+ *vs* IOM − /THC − ($p = 0.022$)
IOM − /THC+	− 60	23	0.04	0.21	
	70	23	6.22	6.56	
	240	23	0.04	0.21	
IOM+/THC+	− 60	23	0.04	0.21	
	70	21	9.14	8.05	
	240	21	0.14	0.48	
CADSS clinician-rated					
IOM − /THC −	− 60	23	0	0	IOM+/THC+ *vs* IOM − /THC − ($p = 0.003$)
	70	23	0.91	1.65	
	240	23	0	0	IOM − /THC+ *vs* IOM − /THC − ($p = 0.06$)
IOM+/THC −	− 60	20	0.05	0.22	
	70	20	1.65	2.16	
	240	20	0	0	
IOM − /THC+	− 60	23	0	0	
	70	23	2.96	3.61	
	240	23	0	0	
IOM+/THC+	− 60	23	0	0	
	70	21	2.95	2.22	
	240	21	0	0	
VAS anxious					
IOM − /THC −	− 60	23	3.62	7.57	IOM+/THC+*vs* THC alone ($p = 0.039$)
	70	23	2.78	8.4	
	240	23	0.32	0.79	IOM+/THC+ *vs* IOM − /THC − ($p = 0.014$)
IOM+/THC −	− 60	20	4.26	9.21	
	70	20	16.37	25.89	IOM+/THC − *vs* IOM − /THC − ($p = 0.053$)
	240	20	1.63	5.4	
IOM − /THC+	− 60	23	3.68	8.94	
	70	23	4.61	8.36	
	240	23	0.3	0.85	
IOM+/THC+	− 60	23	1.72	3.92	
	70	21	16.78	24.82	
	240	21	0.25	0.64	
VAS high					
IOM − /THC −	− 60	23	0.5	2.08	IOM+/THC+ *vs* IOM+/THC − ($p = 0.0001$)
	70	23	7.38	19.12	
	240	23	0.93	2.46	IOM+/THC+ *vs* IOM − /THC − ($p < 0.0001$)
IOM+/THC −	− 60	19	0.42	1.17	
	70	20	11.68	23.08	IOM − /THC+ *vs* IOM+/THC − ($p = 0.004$)
	240	20	0.08	0.18	
IOM − /THC+	− 60	23	0.32	1.06	IOM − /THC+ *vs* IOM − /THC − ($p = 0.0001$)
	70	23	36.32	29.86	
	240	23	0.52	1.2	
IOM+/THC+	− 60	23	0.53	1.69	
	70	21	44.33	33.03	
	240	21	0.38	0.8	

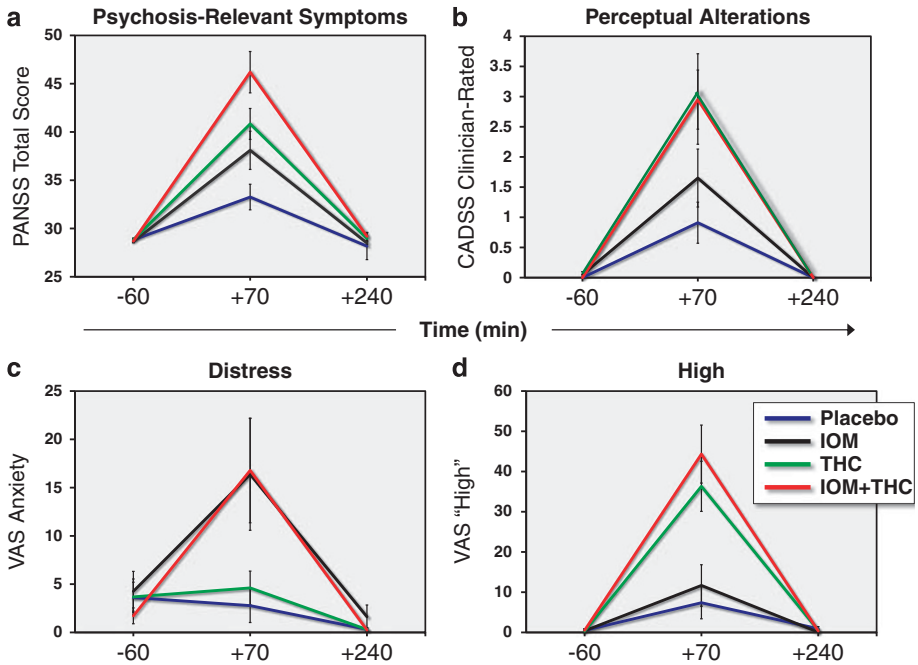

Figure 1 Mean (±SEM) data for each drug condition at baseline (−60 min), +70 min, and +240 min timepoints for the psychosis-relevant symptoms ((a) Total Positive and Negative Syndrome Scale (PANSS)), perceptual alterations ((b) patient-rated Clinician Administered Dissociative Symptoms Scale (CADSS)), distress ((c) VAS Anxiety), and high ((d) VAS High). Placebo, blue line; iomazenil, black line; THC, green line and the combination of iomazenil; and THC: red line. Note that the figure shows mean (SD) data from all timepoints, whereas the analyses were conducted on the peak change from baseline.

combined iomazenil and THC (+IOM+THC), and THC alone (−IOM+THC). Mean (SD) scores on the PANSS (Figure 1), CADSS, and VAS for each drug condition and timepoint are presented in Table 3. Note that even though the statistical analyses were for peak change from baseline, in the figures, *all* timepoints are shown.

Positive and Negative Syndrome Scale. There was a significant drug condition effect ($F_{3,58}=12.93$, $p<0.0001$) on the peak change from baseline in Total PANSS scores. Relative to placebo, the combination of iomazenil and THC (IOM+/THC+) produced significant increases ($p_{adj}<0.0001$) in Total PANSS scores. Relative to placebo, THC alone (IOM−/THC+) produced significant increases ($p_{adj}=0.005$) in Total PANSS scores, while iomazenil did not. Furthermore, there were significantly ($p_{adj}=0.04$) greater increases in Total PANSS scores for the IOM+/THC+ condition compared with IOM−/THC+ (Figure 1a).

Clinician Administered Dissociative Symptoms Scale. There was a significant drug condition effect ($F_{3,59}=5.3$, $p<0.003$) on the peak change from baseline in clinician-rated CADSS subscale scores. However, relative to placebo, only IOM+/THC+ produced a significant increase in the clinician-rated CADSS subscale scores ($p_{adj}=0.003$), while the increase for IOM−/THC+ trended towards significance ($p_{adj}=0.06$). The difference between IOM+/THC+ and IOM−/THC+ was not significant.

There was a significant drug condition effect ($F_{3,58}=5.82$, $p<0.0015$) on the peak change from baseline in patient-rated CADSS subscale scores. Relative to placebo, IOM+/THC+ ($p_{adj}<0.0006$) and IOM−/THC ($p_{adj}=0.02$) produced significant increases in patient-rated CADSS scores (Figure 1b). Although the change in patient-rated CADSS scores was greater on the IOM+/THC+ relative to IOM−/THC (unadjusted $p=0.054$), the difference was not statistically significant after Tukey's adjustment ($p_{adj}=0.2$).

Feeling States (VAS)

'Anxious'. There was a significant drug condition effect ($F_{3,58}=4.82$, $p<0.0046$) on the peak change from baseline in self-reported 'anxious' scores. Relative to placebo, only IOM+/THC+ produced greater increases in VAS 'anxious' scores ($p_{adj}=0.01$) (Figure 1c). Furthermore, IOM+/THC+ produced greater increases in VAS 'anxious' scores relative to IOM−/THC+ ($p_{adj}=0.04$).

'High'. There was a significant drug condition effect ($F_{3,57}=14.27$, $p<0.0001$) on the peak change from baseline in self-reported 'high' or 'stoned' scores. Relative to placebo, both IOM−/THC+ ($p_{adj}=0.0001$) and IOM+/THC+ ($p_{adj}<0.0001$), but not iomazenil alone, produced significant increases in VAS 'high' scores (Figure 1d). Furthermore, there were no significant differences between the IOM−/THC+ and IOM+/THC+ conditions.

EEG Measures

P300b. Relative to placebo, only IOM+/THC+ produced a significant ($p=0.023$) decrease in target P300b amplitude at Pz (Figure 2; Table 4). Furthermore, there was a significant linear trend ($F_{1,44}=6.02$, $p=0.018$) for drug condition (IOM−/THC−>IOM+/THC−>IOM−/THC+>IOM+/THC+). There were no significant effects of any of the drug conditions on target P300b latency measured at Pz (Table 4).

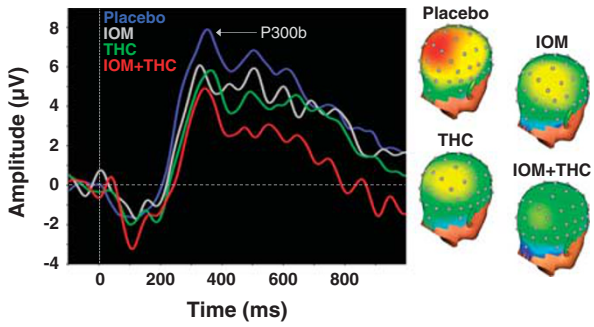

Figure 2 (Left) Grand-averaged target P300b waveforms at electrode Pz for EEG runs across drug conditions. (Right) Topographic voltage maps from the peak grand-averaged P300b for EEG runs across drug conditions. Placebo, blue line; iomazenil, gray line; THC, green line and the combination of Iomazenil; and THC, red line.

P300a. Relative to IOM−/THC−, both IOM+/THC+ and IOM−/THC+ produced trend level ($p=0.08$) reductions in P3a amplitude measured at Cz. Furthermore, there was a trend towards a linear trend ($F_{1,44}=3.11$, $p=0.085$) for drug condition (IOM−/THC− > IOM+/THC− > IOM−/THC+ > IOM+/THC+). Relative to IOM−/THC−, only IOM+/THC+ produced a trend levels reduction in ($p=0.08$) P300a latency measured at Cz (Table 4).

There were no correlations between the effects of IOM+/THC+ or IOM−/THC+ on PANSS outcomes and both P3b and P3a amplitudes (Supplementary Table 1).

N100. There were no significant effects of THC, IOM, or their combination on N100 amplitude or latency for both target and novel stimuli measured at Cz.

Safety

There were no serious adverse events that occurred on test days. Prospective poststudy safety assessments (described in Supplementary Text no. 3) did not reveal any evidence, suggestive of an increase in cannabis use or psychosis outcomes.

DISCUSSION

This is the first study to our knowledge that examines the effects of THC in the backdrop of a pharmacologically induced GABA deficit in humans. The results showed that pretreatment with IOM exacerbated several of the behavioral, subjective, and psychophysiological effects of THC in healthy humans. When pretreated with IOM, THC induced significantly greater psychosis-relevant symptoms, as captured by the Total PANSS, compared with the THC-alone condition. Similarly, only with IOM pretreatment did THC significantly increase perceptual alterations as captured by the patient-rated CADSS. Furthermore, only when pretreated with IOM did THC induce distress as captured by VAS 'anxiety' scores. Finally, only the combination of IOM+THC reduced THC-induced P300b amplitude.

The combination of IOM and THC did not increase measures of euphoria (VAS 'high') compared with THC

Table 4 Electrophysiological Effects of THC and Iomazenil

Condition	*N*	Mean	SD	Only significant contrasts
P3b amplitude (Pz)				
IOM−/THC−	17	10.23	5.81	+IOM+THC *vs* PLA+PLA: DF=44, *t*-value=2.34, $p=0.024$
IOM+/THC−	16	9.24	6.32	
IOM−/THC+	19	8.33	6.36	
IOM+/THC+	16	7.73	5.48	
P3b latency (Pz)				
IOM−/THC−	17	328.97	35.8	
IOM+/THC−	16	325.46	26.95	
IOM−/THC+	19	342.17	21.38	
IOM+/THC+	16	327.55	24.69	
P3a amplitude (Cz)				
PLA+PLA	17	11.18	7.16	IOM+THC *vs* PLA+PLA: DF=44, *t*-value=0.18, $p=0.08$
IOM+PLA	16	8.55	4.56	PLA+THC *vs* PLA+PLA: DF=44, *t*-value=1.79, $p=0.08$
PLA+THC	19	7.79	6.4	
IOM+THC	16	8.29	5.36	
P3a latency (Cz)				
IOM−/THC−	17	325.98	23.2	
IOM+/THC−	16	320.92	29.03	
IOM−/THC+	19	322.18	28.5	
IOM+/THC+	16	312.96	18.46	
Target N100 amplitude (Cz)				
IOM−/THC−	17	−5.97	3.53	
IOM+/THC−	16	−5.56	3.32	
IOM−/THC+	19	−5.37	2.38	
IOM+/THC+	16	−6.99	3.86	
Target N100 latency (Cz)				
IOM−/THC−	17	103.36	11.06	
IOM+/THC−	16	107.38	16.77	
IOM−/THC+	19	108.95	14.01	
IOM+/THC+	16	109.3	13.59	
Novel N100 amplitude (Cz)				
IOM−/THC−	17	−6.13	4	
IOM+/THC−	16	−5.89	3.93	
IOM−/THC+	19	−5.36	2.55	
IOM+/THC+	16	−5.91	2.9	
Novel N100 latency (Cz)				
IOM−/THC−	17	107.12	14.24	
IOM+/THC−	16	111.94	10.34	
IOM−/THC+	19	106.64	18.48	
IOM+/THC+	16	106.36	12.56	

alone. Furthermore, there were no differences in THC blood levels across conditions. Taken together, these findings suggest a pharmacodynamic rather than a pharmacokinetic interaction.

The close interplay between the CB and GABAergic systems in several brain regions provides a mechanistic framework to understand the study findings. In the cerebral cortex and hippocampus, presynaptic CB1-Rs primarily inhibit the release of GABA from CCK− interneurons (Eggan *et al*, 2010). IOM likely causes a net reduction in $GABA_A$ function across various interneuron types (eg, PV, SST, CCK, and so on). However, as PV-positive interneurons are thought to be the primary GABAergic subtype involved in psychosis (Glausier *et al*, 2014; Lewis *et al*, 2012), it is tempting to speculate if IOM exacerbates the psychotomimetic effects of THC by disrupting inputs from PV cells onto PCs. Indeed, several lines of evidence suggest that CB1-R-positive CCK interneurons and PV cells work in concert to modulate the synchronized output of PCs (Bartos and Elgueta, 2012; Klausberger *et al*, 2005). Put another way, THC-induced activation of CB-1Rs on the axon terminals of CCK containing GABA neurons reduces GABA release, resulting in disinhibition of PC activity. If this were to occur in the presence of a GABA deficit, as might be produced by IOM (and as might occur in schizophrenia), it would lead to further disinhibition and desynchronization of PC activity, which would lead to the perturbation of gating and associative functions and culminate in psychotic symptoms.

Pretreatment with IOM has been shown to enhance the psychotomimetic effects of serotonergic (*m*-CPP) (D'Souza *et al*, 2006), dopaminergic (amphetamine) (K Ahn, personal communication), glutamatergic (ketamine) (H Gunduz-Bruce, personal communication), and now cannabinoidergic (THC) agents. Furthermore, when administered alone, IOM has been shown to produce small increases in psychosis in schizophrenia patients but not in healthy controls (Ahn *et al*, 2011). Collectively, these findings highlight the contributions of GABAergic deficits to the pathophysiology of schizophrenia.

The combination of IOM and THC also caused the largest reductions in the P300b. Exogenous cannabinoids have been shown to reduce the amplitude of the P300b (D'Souza *et al*, 2012; Roser *et al*, 2008). Reductions in P300 amplitude and increased latencies have been observed in a number of other neuropsychiatric disorders including schizophrenia (reviewed in Bramon *et al*, 2004; Jeon and Polich, 2003). Both GABAergic and glutamatergic systems have been strongly implicated in contributing to the P300 (Watson *et al*, 2009). In fact, it is now recognized that ERPs such as the P300 are generated by inhibitory and excitatory postsynaptic potentials, which are primarily driven by the release of glutamate and GABA (Luck *et al*, 2011). It is likely that normal P300 generation requires an optimal level of inhibitory–excitatory balance, and any perturbation above or below the optimum range can disrupt the neural networks involved in context updating, working memory, and the allocation of attentional resources. Future research is needed to determine how GABA-CB effects on the P300 are related to the psychosis-enhancing effects of IOM and THC.

These results lend support to the hypothesis that a pharmacologically induced GABA deficit would enhance the psychosis-relevant effects of THC in healthy adults. These data suggest that the enhanced vulnerability to cannabis and THC in schizophrenia patients (D'Souza *et al*, 2005) may be explained by underlying GABA deficits. Although admittedly speculative, it may be inferred that psychosis-prone individuals who appear sensitive to the psychosis-inducing effects of cannabis may also have GABAergic deficits. However, to our knowledge, the functional state of the GABA system has not been examined in psychosis prone individuals.

These data add to a growing body of evidence from epidemiologic and experimental studies that have identified other factors that modulate the acute response to cannabis and THC, respectively. Caspi *et al,* (2005) demonstrated that polymorphisms of the gene encoding catechol-*O*-methyltransferase (*COMT*), which is critical for the removal of dopamine (DA) in the prefrontal cortex, influenced the risk for psychosis outcomes in later life, following cannabis exposure in adolescence. Henquet *et al*, (2006) then showed in an experimental study that polymorphisms of the gene encoding *COMT*-mediated differential sensitivity to the acute psychotomimetic effects of THC (Henquet *et al*, 2006). Similarly, epidemiologic studies have shown that variation in the gene for protein kinase C (AKT1), an integral component of the DA signaling cascade, influences the risk of associated psychosis outcomes with cannabis use (Di Forti *et al*, 2012; van Winkel *et al*, 2011a; van Winkel *et al*, 2011b). In an experimental study, Bhattacharyya *et al*, (2012) showed that polymorphisms of the genes for AKT1 and the dopamine transporter (DAT1) influence the psychotomimetic and neurophysiological response to THC. Finally, in a recent study, Bhattacharyya *et al*, (2014) also showed that the AKT1 genotype mediates the sensitivity to THC-induced impairments in psychomotor control.

Strengths, Limitations, and Conclusions

The intravenous route of drug administration, and weight-adjusted doses address the inter- and intraindividual variability associated with oral or smoked THC. The randomized, placebo-controlled, double-blind, repeated-measure, 2 (active or placebo THC) ×2 (active or placebo iomazenil), within-subjects design is both powerful and efficient. The behavioral and cognitive outcome measures were complemented by a psychophysiological measure that allows a more proximal index of neuronal activity and interaction of CB and GABA systems. Further, ERPs afford near-perfect temporal resolution that is not afforded by other approaches. However, while the proposed study may have limited social relevance, as cannabis is not typically used intravenously, the strengths of the intravenous paradigm outweigh its limited social relevance. Finally, the study was not powered to evaluate interactive effects on all the subscales of the outcome measures or the influence of cannabis exposure on the interactions between IOM and THC.

Future Directions

Whether these findings can be generalized to women needs further study. These findings need replication in a larger sample that is adequately powered to examine the outcome measures in further detail, for example, items of the PANSS.

The animal literature shows that GABA and CB systems modulate gamma range (γ)-band neural oscillations, which are thought to have a key role in a number of processes that are altered in schizophrenia (Uhlhaas *et al*, 2009), including sensory registration, the integration and binding of perceptual features, associative learning, and conscious awareness; it will be important to study the interplay between CB and GABA systems on (γ)-band neural oscillations in humans. The development and availability of reliable and valid *in vivo* methods to determine GABAergic deficits in schizophrenia will permit a more direct approach to determining the contribution of GABAergic deficits to vulnerability to the psychosis-inducing effects of THC in schizophrenia. Finally, the availability of drugs that target specific GABA interneurons will permit a more refined testing of the hypothesis.

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