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Stress-Induced Dopamine Response in Subjects at Clinical High Risk for Schizophrenia with and without Concurrent Cannabis Use

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Research on the environmental risk factors for schizophrenia has focused on either psychosocial stress or drug exposure, with limited investigation of their interaction. A heightened dopaminergic stress response in patients with schizophrenia and individuals at clinical high risk (CHR) supports the dopaminergic sensitization hypothesis. Cannabis is believed to contribute to the development of schizophrenia, possibly through a cross-sensitization with stress. Twelve CHR and 12 cannabis-using CHR (CHR-CU, 11 dependent) subjects underwent [^{11}C]-(+)-PHNO positron emission tomography scans, while performing a Sensorimotor Control Task (SMCT) and a stress condition (Montreal Imaging Stress task). The simplified reference tissue model was used to obtain binding potential relative to non-displaceable binding (BP_{ND}) in the whole striatum, its functional subdivisions (limbic striatum (LST), associative striatum (AST), and sensorimotor striatum (SMST)), globus pallidus (GP), and substantia nigra (SN). Changes in BP_{ND}, reflecting alterations in synaptic dopamine (DA) levels, were tested with analysis of variance. SMCT BP_{ND} was not significantly different between groups in any brain region ($p > 0.21$). Although stress elicited a significant reduction in BP_{ND} in the CHR group, CHR-CU group exhibited an increase in BP_{ND}. Stress-induced changes in regional BP_{ND} between CHR-CU and CHR were significantly different in AST ($p < 0.001$), LST ($p = 0.007$), SMST ($p = 0.002$), SN ($p = 0.021$), and whole striatum ($p = 0.001$), with trend level in the GP ($p = 0.099$). All subjects experienced an increase in positive (attenuated) psychotic symptoms ($p = 0.001$) following the stress task. Our results suggest altered DA stress reactivity in CHR subjects who concurrently use cannabis, as compared with CHR subjects. Our finding does not support the cross-sensitization hypothesis, which posits greater dopaminergic reactivity to stress in CHR cannabis users, but adds to the growing body of literature showing reduced DA (stress) response in addiction.

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INTRODUCTION

Cannabis is the most widely used illicit substance around the world (Bauman and Phongsavan, 1999) and is the illicit drug most commonly used by people with psychosis (Fowler *et al*, 1998; Hafner *et al*, 1999; Menezes *et al*, 1996), and those at elevated clinical risk for schizophrenia (Rosen *et al*, 2006). Several epidemiological studies have found that cannabis use increases the likelihood of developing schizophrenia (and psychosis) 1.8- to 3.1-fold (Andreasson *et al*, 1987; Arseneault *et al*, 2002, as reviewed in Arseneault *et al*, 2004). However, little is known about its effects on brain neurochemistry, or its impact on dopamine (DA) transmission, which is important as schizophrenia presents with abnormal DA synthesis and release (Laruelle and Abi-Dargham, 1999).

Schizophrenia is now perceived as a complex multi-factorial disorder in which genetic predisposition and environmental factors interact to cause the disease. Both cannabis (Thornicroft, 1990) and stress (Norman and Malla, 1993) can exacerbate pre-existing psychotic symptoms or trigger their re-emergence in some (but not all) individuals with psychotic-related disorders (Mathers and Ghodse, 1992; Negrete *et al*, 1986; Thornicroft, 1990). A dysregulated response to stress and cannabis has been proposed as a potential etiological factor in the development of schizophrenia and its relapse. This model suggests that an endogenous organic diathesis or vulnerability interacts with internal or external stressors or drugs in the development of psychotic disorders (Murray and Fearon, 1999), with a number of environmental risk factors such as social

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alienation, early-life adversity, and cannabis use exerting a much higher effect on a sensitive subgroup (van Os *et al*, 2011). Although the underlying neurobiological condition/event that leads to increased vulnerability and causes exaggerated responses to stressors or drugs is unknown, one proposed mechanism is DA sensitization, whereby repeated exposure to life stressors or drugs progresses into increased stress (or drug)-associated DA activity, thus precipitating psychosis in those at risk or relapse in patients (Laruelle and Abi-Dargham, 1999; van Os *et al*, 2005). Indeed, recent work has shown increased DA release in response to psychosocial stress in individuals at clinical high risk (CHR) of developing schizophrenia and in antipsychotic-naive patients with schizophrenia (Mizrahi, 2010; Mizrahi *et al*, 2012). However, no such effect was observed in nonpsychiatric chronic cannabis users (Mizrahi *et al*, 2013) or healthy volunteers with no past history of low maternal care (Pruessner *et al*, 2004).

Interestingly, cannabinoids produce behavioral as well as neurochemical changes dependent on the environmental conditions under which they are administered. For example, cannabinoids administered to rats housed in stressful conditions alter striatal DA uptake and metabolism in contrast to the absence of effect on rats housed in normal conditions (Littleton *et al*, 1976; MacLean and Littleton, 1977). Furthermore, cross-sensitization between Δ^9-tetrahydrocannabinol (THC) and stress has been reported (Suplita *et al*, 2008), suggesting that the physiological and psychological effects of cannabis could be altered in individuals experiencing environmental adversity. Indeed, recent studies in humans have shown that levels of the stress hormone cortisol correlate with the magnitude of DA release in response to amphetamine (Oswald *et al*, 2005). In addition, risk of psychosis has been shown to increase with childhood trauma and cannabis use through a synergistic interaction (Harley *et al*, 2010).

Positron emission tomography (PET) imaging provides the means of estimating changes in DA concentrations *in vivo*. Endogenous DA competes with the radiotracer for binding to the $D_{2/3}$ receptors in the brain reducing the measured radiotracer binding potential (as reviewed in Laruelle, 2000). [^{11}C]-(+)-PHNO, a $D_{2/3}$ receptor agonist radiotracer used in this study, binds with $\sim$20-fold higher affinity for D_3 over D_2 receptor, providing increased sensitivity and allowing for quantification of the D_3 receptor subtype (Narendran *et al*, 2006; Rabiner and Laruelle, 2010). In recent years, the role of DA D_3 receptors in the neurochemical changes associated with drug dependence and relapse has come under intense investigation (Heidbreder *et al*, 2005; Ikemoto and Panksepp, 1999), with proposals of using D_3-selective inhibitors for treatment of substance dependence (Heidbreder and Newman, 2010).

On the basis of the potential cross-sensitization between stress and cannabis in those at risk of developing schizophrenia, and the well-known finding of increased incidence of schizophrenia with early cannabis use, we tested the hypothesis that individuals at CHR of developing schizophrenia with concurrent cannabis use (cannabis-using CHR (CHR-CU)) have greater dopaminergic responses (increased [^{11}C]-(+)-PHNO displacement) to a validated psychosocial stress challenge (Pruessner *et al*, 2004), as compared with CHR individuals with no cannabis use.

MATERIALS AND METHODS

Subjects

All subjects completed two PET scans at the same time of the day at least a week apart, first while performing a Sensory Motor Control Task (SMCT) and second the Montreal Imaging Stress Task (MIST). Data on [^{11}C]-(+)-PHNO imaging with CHR subjects (but not CHR-CU) were used from a previous study (Mizrahi *et al*, 2012). CHR-CU were asked to refrain from using cannabis on the day of the scan (information on hours since the last use is provided below). All subjects were scanned during the same time frame and recruited from the same geographical region.

Inclusion criteria were as follows: (1) men or women between 18 and 40 years old; (2) capacity to provide informed consent; (3) meet diagnostic criteria for prodromal syndrome as per the Criteria of Prodromal Syndromes (COPS); (4) 'moderately ill' on the Clinical Global Impression Scale or significant impairment in functioning, ie, <52 on Global Assessment of Functioning (GAF) scale (Miller *et al*, 2003) or >9 on Scale of Prodromal Symptoms-positive subscale (SOPS-P). In addition, for CHR-CU, (5) regular cannabis use at least three times weekly and/or meeting DSM-IV criteria for cannabis dependence, and (6) positive drug screen for cannabis both at screening and on the days of the PET scans. Exclusion criteria were as follows: (1) current or lifetime Axis I psychotic disorder, including affective psychoses (excluding cannabis dependence in CHR-CU); (2) current treatment with antipsychotic medication or lifetime use >4 weeks; (3) past or current history of a clinically significant central nervous system disorder that may contribute to prodromal symptoms or confuse their assessment; (4) substance abuse or dependence in the past 6 months (excluding cannabis for the CHR-CU group); and (5) metal implants that would preclude magnetic resonance imaging (MRI).

Assessment

Psychopathology measures. CHR were classified as per the COPS using the Structured Interview for Prodromal Symptoms (SIPS), which was administered to assess attenuated psychotic symptoms (McGlashan *et al*, 2001; Miller *et al*, 2002), incorporating the family history, GAF, and schizotypal personality disorder information. The CHR criteria include the following: attenuated positive symptoms syndrome, the genetic risk and deterioration syndrome, and the brief intermittent psychosis syndrome. The SOPS, which is part of the SIPS, is a dimensional 19-item instrument for quantifying prodromal state severity and has been used with SIPS to identify COPS criteria with excellent inter-rater reliability ($\kappa = 0.81$; Miller *et al*, 2003). These results give a positive predictive value of 54%, a psychotic/nonpsychotic sensitivity of 1.0, and a specificity of 0.73 (Miller *et al*, 2003), supporting the criteria's validity for defining prodromal states that mark high imminent risk for psychosis. In addition, all subjects were screened for any Axis I psychopathology with the Structured Clinical Interview for DSM-IV by a qualified psychiatrist (RM), and for marijuana use with the Marijuana Craving Questionnaire (MCQ) scale (Heishman and Singleton, 2006) and detailed personal and medical history.

Montreal Imaging Stress Task

Psychological stress was induced using the MIST task, which has been validated in previous fMRI and PET studies (Booij *et al*, 2007; Dedovic *et al*, 2005; Lederbogen *et al*, 2011; Pruessner *et al*, 2004; Pruessner *et al*, 2007). Briefly, subjects perform six 6-min segments of arithmetic while lying in the scanner. During the stress condition, the time constraint is adjusted to be slightly beyond each individual's abilities. Subjects were given negative verbal feedback by the investigator between each block, telling them that they need to improve their performance to reach minimum performance requirements. Before the stress task, subjects performed the sensory motor control PET session, a similar arithmetic task without time constraints or negative verbal feedback. In all experiments, the control or stress task was started $\sim$6–8 min before tracer injection, with 6 min of arithmetic questions and $\sim$1 to 2 min for either neutral or negative feedback and salivary cortisol measurement. The non-stress control was also administered as a practice trial on a separate day before the PET experiments, to reduce the effect of novelty. Subjective perception of stress was assessed before and after each PET session by state anxiety questionnaires (SAQs) (Spielberger *et al*, 1977) and visual analog scales. Subjects also completed the Parental Bonding Index (Parker *et al*, 1979), which has been associated with DA release in healthy volunteers (Pruessner *et al*, 2004).

Physiological Measures

Saliva samples were collected every 12 min throughout the experiment. Saliva-derived cortisol was analyzed using a time-resolved fluorescence immunoassay (Dressendorfer *et al*, 1992) and the area under the curve (AUC; g/dl/min) was calculated for each scanning session (Dressendorfer *et al*, 1992; Pruessner *et al*, 2003).

Image and Data Analyses

MRI acquisition. Subjects undertook a standard fast spin echo T1 (FSPGR, TE = 5.3–15, TR = 8.9–12, FOV = 20 cm, matrix = 256 × 256, slice thickness = 1.5, NEX = 1) and a proton density (TE = 17, TR = 6000, FOV = 22 cm, matrix = 256 × 256, slice thickness = 2 mm, NEX = 2) brain MRI images acquired on a 1.5T Signa-GE scanner. These images were used for the analysis of the individual PET scans and to rule out structural lesions.

PET acquisition. [^{11}C]-(+)-PHNO radiosynthesis was performed as previously described (Wilson *et al*, 2005). Studies were carried out using a high-resolution PET/CT Siemens-Biograph HiRez XVI scanner (Siemens Molecular Imaging, Knoxville, TN), which measures radioactivity in 81 brain sections with a thickness of 2.0 mm each. PET data was acquired for 90 min following administration of $\sim$9–10 mCi of radiotracer (Table 1). A custom-fitted thermoplastic mask was made for each subject and used with a head fixation system during PET measurements to minimize head movement. The images were reconstructed with 2D filtered back projection algorithms with a ramp filter at Nyquist cut-off frequency.

PET data analysis. Time activity curves from the regions of interest (ROIs) were obtained from the dynamic [^{11}C]-(+)-PHNO PET images. The striatum was divided using the individual subject's MRI into subdivisions based on the functional connections to the limbic, frontal executive, and motor brain regions: limbic striatum (LST, including the ventral striatum), associative striatum (AST, including the pre-dorsal putamen, and pre-dorsal and post-dorsal caudate), and sensorimotor striatum (SMST, post-dorsal putamen), based on a set of landmarks as described previously (Martinez *et al*, 2003). We also report stress-induced changes in non-displaceable binding (BP_{ND}) in the globus pallidus (GP) and substantia nigra (SN), using ROI landmarks previously described (Tziortzi *et al*, 2011). The ROIs were delineated using an automated method implemented in an in-house software (ROMI), abolishing subjectivity in manual ROI drawing (Rusjan *et al*, 2006). Activity from the right and left regions were averaged together, weighted by subregion volume, and used to derive binding potential of the radiotracer with respect to the non-displaceable compartment (BP_{ND}) using the simplified reference tissue model (SRTM). BP_{ND} is proportional to the more fundamental parameters of receptor number (B_{max}) and affinity ($1/K_d$) [$BP \approx B_{max}/K_d$]. This method has been validated for BP_{ND} with [^{11}C]-(+)-PHNO (Ginovart *et al*, 2007). Partial volume effects were corrected using the method of Rousset *et al* (1998), with further details provided in the online Supplementary Data.

Voxel-wise images were generated using the Receptor Parametric Mapping software (Gunn *et al*, 1997), where the subregion of the cerebellar cortex (excluding the vermis) almost completely devoid of $D_{2/3}$ receptors served as the reference region. Each parametric map was spatially normalized to an anatomical template (Montreal Neurological Institute) using Statistical Parametric Mapping (SPM) normalization and coregistration tools. BP_{ND} maps were used to assess significant contrast between conditions as follows: between groups for the baseline SMCT with independent *t*-test and SMCT *vs* MIST paired *t*-test within each group (CHR and CHR-CU) at the voxel level using an implicit mask of $BP_{ND} > 0.3$ as reported previously (Mizrahi *et al*, 2012). Family-wise error correction was used as implemented in SPM2 (www.fil.ion.ucl.ac.uk/spm).

Statistical Analysis

The primary hypothesis was tested using analysis of variance (ANOVA) to investigate differences in stress-induced [^{11}C]-(+)-PHNO % BP_{ND} change between CHR and CHR-CU subjects, defined as $\%\ \text{change in}\ BP_{ND} = \frac{BP_{ND}\ SMCT - BP_{ND}\ MIST}{BP_{ND}\ SMCT} \times 100\ \%$. Subjective perceived stress and cortisol stress levels were compared using ANOVAs between conditions (SMCT and MIST). Linear regression analyses were used to test the associations between stress-induced DA release and psychopathology. All analyses are two tailed with the conventional $\alpha = 0.05$.

Table 1 No Significant Difference between Groups or Conditions was Observed in Any Variable, Except for Smoking Status ($\chi^2 = 8.167$, $p = 0.004$) and Trend Level for the Maternal Portion of the PBI ($F = 3.323$, $df = 1,22$, $p = 0.082$)

Demographics (SD)	CHR $n = 12$	CHR-CU $n = 12$	Comparison
Age (years)	23.00 ± 4.6	24.25 ± 4.7	$F = 0.419$, $df = 1,22$, $p = 0.524$
Gender			
Male	7	6	$\chi^2 = 0.167$, $p = 0.683$
Female	5	6	
Mother PBI	35.83 ± 5.8	40.42 ± 6.5	$F = 3.323$, $df = 1,22$, $p = 0.082$
Smoking status			
Non-smoker	11	8	$\chi^2 = 8.167$, $p = 0.004$
Smoker	1	4	
Cigarettes/day	10	9.13 ± 8.0	
Years of education	14.08 ± 3.1	13.25 ± 1.8	$F = 0.631$, $df = 1,22$, $p = 0.435$
Lifetime use (joints)	NA	4892.91 ± 4100.2	
Years of cannabis use	NA	9.64 ± 4.8	
Age at first cannabis use (years)	NA	14.82 ± 2.7	
Number of joints per week			
Control task	NA	17.00 ± 7.3	
Stress task	NA	14.36 ± 7.8	
Hours since last joint smoked			
Control task	NA	11.00 ± 5.3	
Stress task	NA	13.3 ± 5.8	
Cannabinoids value (μg/l)			
Control task	NA	1852.81 ± 2909.7	
Stress task	NA	2730.20 ± 3347.4	
SOPS symptoms			
Positive	11.25 ± 2.9	12.64 ± 2.0	$F = 1.771$, $df = 1,21$, $p = 0.198$
Negative	10.17 ± 5.3	6.18 ± 3.6	$F = 4.356$, $df = 1,21$, $p = 0.049$
Disorganized	4.17 ± 2.1	4.36 ± 2.5	$F = 0.042$, $df = 1,21$, $p = 0.840$
General	5.25 ± 3.2	4.36 ± 3.5	$F = 0.410$, $df = 1,21$, $p = 0.529$
PET scan parameters			
Amount injected (mCi)			
Control task	9.17 ± 2.2	9.98 ± 1.1	$F = 1.325$, $df = 1,22$, $p = 0.262$
Stress task	9.89 ± 0.96	9.69 ± 0.99	$F = 0.247$, $df = 1,22$, $p = 0.624$
Specific activity (mCi/μmol)			
Control task	1014.84 ± 434.3	1199.83 ± 569.2	$F = 0.801$, $df = 1,22$, $p = 0.380$
Stress task	1003.82 ± 470.6	1484.04 ± 628.2	$F = 4.492$, $df = 1,22$, $p = 0.046$
Mass injected (μg)			
Control task	2.58 ± 0.90	2.26 ± 0.96	$F = 0.743$, $df = 1,22$, $p = 0.398$
Stress task	2.92 ± 0.90	1.93 ± 0.90	$F = 7.255$, $df = 1,22$, $p = 0.013$

Abbreviations: CHR, clinical high; CHR-CU, cannabis-using CHR; NA, not applicable; PBI, Parental Bonding Index; PET, positron emission tomography; SOPS, Scale of Prodromal Symptoms.

RESULTS

A total of 48 PET scans were acquired for the present study. CHR ($n = 12$) and CHR-CU ($n = 12$) groups had comparable demographics (Table 1). Out of 12 CHR-CU subjects, 11 met criteria for cannabis dependence and exhibited daily or higher use for at least 2 years (Table 1). Four subjects had no exposure to other drugs, whereas the remaining eight reported past recreational use (with only five using drugs in the past 12 months before scanning), with no abuse or

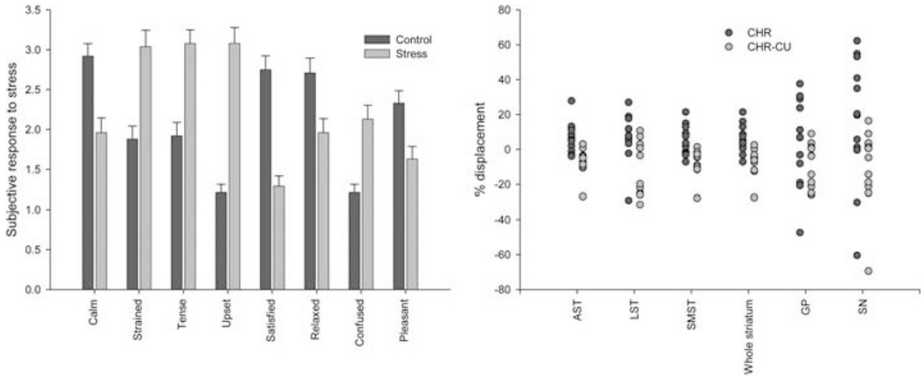

Figure 1 Left: subjective response to stress for all subjects, depicting mean pre- *vs* post-scan change in State Anxiety Questionnaire (SAQ) categories (SE). Right: [^{11}C]-(+)-PHNO positron emission tomography (PET) response to the stress in different regions of the brain.

dependence, of cocaine ($n=6$), amphetamine ($n=1$), ecstasy ($n=5$), mushrooms ($n=2$), LSD ($n=2$), ketamine ($n=1$), opioids ($n=2$), and methylphenidate ($n=1$). Within the CHR group, all subjects reported no history of drug use in the last 12 months before scanning, except for one subject who admitted to using cannabis three to five times per month (but had a negative drug screen on scanning days). At the time of the scan, urine drug screen confirmed lack of other substance abuse in all subjects, except for cannabis both at screening and on PET scan days, detected only in the CHR-CU group. None of the subjects exhibited any Axis I conditions at the time of the scan, except for cannabis dependence in the CHR-CU group. In the CHR group, one subject used fluoxetine (20 mg/day) and one used clonazepam (0.5 mg/day), whereas another one of the CHR-CU subjects used sertraline (100 mg/day).

All subjects performed significantly worse at the MIST (number of errors 34.82 ± 10.7 and 35.11 ± 5.9 for CHR and CHR-CU, respectively), compared with the SMCT (5.17 ± 3.4 and 6.40 ± 3.0 for CHR and CHR-CU, respectively; paired sample *t*-test between conditions $t=-9.96$, $p<0.001$ for CHR and $t=-15.15$, $p<0.001$ for CHR-CU). CHR and CHR-CU subjects did not differ in their performance, quantified as number of errors and timeouts, either in the control ($F=0.90$, $df=1,22$, $p=0.353$) or stress task ($F=0.054$, $df=1,21$, $p=0.82$). As expected, SAQs revealed that all subjects were less calm ($F=15.46$, $df=1,46$, $p<0.001$) and satisfied ($F=46.11$, $df=1,46$, $p<0.001$), but more tense ($F=23.73$, $df=1,46$, $p<0.001$), strained ($F=19.99$, $df=1,46$, $p<0.001$), upset ($F=69.83$, $df=1,46$, $p<0.001$), and confused ($F=20.54$, $df=1,46$, $p<0.001$) following the stress task than following the control task, supporting the effectiveness of the stress paradigm (Figure 1, left panel). Comparing the differences between post-SMCT and post-MIST SAQ values, CHR-CU reported feeling more strained ($F=7.00$, $df=1,22$, $p=0.015$), tense ($F=7.59$, $df=1,22$, $p=0.012$), upset ($F=18.267$, $df=1,22$, $p<0.001$), and less satisfied ($F=7.406$, $df=1,22$, $p=0.012$) than the CHR subjects (additional behavioral information presented in the Supplementary Material). All subjects showed an increase in psychotic-like experiences following the stress task as opposed to the control task ($t=-4.63$, $df=47$, $p<0.001$). Both CHR and CHR-CU showed an increase in positive SOPS following the stress task (CHR: $t=-2.292$, $df=11$, $p=0.043$; CHR-CU: $t=-3.527$, $df=11$, $p=0.005$), but not the control task (CHR: $t=-1.241$, $df=11$, $p=0.241$; CHR-CU: $t=-1.820$, $df=11$, $p=0.096$), relative to the assessment at screening. Interestingly, although no significant difference was observed between the CHR- and CHR-CU-positive and disorganized SOPS scores at baseline screening, CHR-CU exhibited significantly less negative symptoms compared with CHR ($F=4.356$, $df=1,21$, $p=0.049$; Table 1). Comparing pre- and post-scan SOPS, significant increases were observed in positive attenuated symptoms following SMCT and MIST scans in the CHR group (SMCT: $t=2.283$, $df=11$, $p=0.043$; MIST: $t=2.754$, $df=11$, $p=0.019$) but only following the MIST in CHR-CU group (SMCT: $t=1.915$, $df=11$, $p=0.082$; MIST: $t=3.079$, $df=11$, $p=0.010$).

BP_{ND} data did not show any difference between CHR and CHR-CU in any brain region investigated during the control task (Table 2): AST ($F=0.22$, $df=1,22$, $p=0.644$), LST ($F=0.17$, $df=1,22$, $p=0.687$), SMST ($F=0.55$, $df=1,22$, $p=0.468$), the whole striatum ($F=0.11$, $df=1,22$, $p=0.743$), GP ($F=1.6$, $df=1,22$, $p=0.219$), and SN ($F=0.03$, $df=1,22$, $p=0.870$). However, we found a significant difference in stress-induced %change in [^{11}C]-(+)-PHNO BP_{ND} in CHR-CU relative to CHR in the entire striatum (Table 2; $F=16.60$, $df=1,22$, $p=0.001$), its subdivisions (AST: $F=17.90$, $df=1,22$, $p<0.001$), LST: $F=9.03$, $df=1,22$, $p=0.007$, and SMST: $F=11.67$, $df=1,22$, $p=0.002$), and SN ($F=6.22$, $df=1,22$, $p=0.021$), with a trend level in the GP ($F=2.97$, $df=1,22$, $p=0.099$). These findings present robust Cohen's d effect sizes of -1.61 (AST), -1.28 (LST), -1.32 (SMST), -1.56 (whole striatum), -0.55 (GP), and -0.77 (SN). Applying Bonferroni correction for multiple comparisons, results remain significant in all regions, except for SN. Same results are obtained when the mass of [^{11}C]-(+)-PHNO was added as a covariate to the analysis, with the exception of the difference in %change in SN, which becomes almost significant ($F=3.172$, $p=0.063$) but remains in the same direction. Differences in %change remain significant following partial volume effect correction, with the exception of the SN ($F=1.638$, $df=1,22$, $p=0.214$). The results also hold when the participants who used tobacco are excluded, with differences in %change in LST and SN becoming trend level: $F=4.404$, $p=0.051$ and $F=3.624$, $p=0.074$, respectively. In addition, even when the subjects

Table 2 BP_{ND} for Each Brain Region Studied in the Control Task (SMCT) and Stress Task (MIST) for Each Group

Regions	CHR				CHR-CU			
	BP_{ND} SMCT	BP_{ND} MIST	% Change	Paired *t*-test (df = 11)	BP_{ND} SMCT	BP_{ND} MIST	% Change	Paired *t*-test (df = 11)
AST	2.45 ± 0.6	2.28 ± 0.5	6.97 ± 8.7	$t = 2.39$ $p = 0.036$	2.36 ± 0.4	2.52 ± 0.4	− 7.04 ± 7.5	$t = -3.66$ $p = 0.004$
LST	2.79 ± 0.7	2.55 ± 0.5	7.20 ± 13.8	$t = 2.08$ $p = 0.062$	2.89 ± 0.4	3.17 ± 0.5	− 10.48 ± 15.0	$t = -2.33$ $p = 0.040$
SMST	2.64 ± 0.6	2.45 ± 0.5	4.55 ± 8.7	$t = 2.51$ $p = 0.029$	2.49 ± 0.4	2.65 ± 0.4	− 7.00 ± 7.8	$t = -3.44$ $p = 0.006$
Whole striatum	2.53 ± 0.5	2.35 ± 0.5	6.32 ± 8.8	$t = 2.35$ $p = 0.039$	2.47 ± 0.4	2.63 ± 0.4	− 7.35 ± 7.6	$t = -3.679$ $p = 0.004$
GP	2.51 ± 1.5	2.21 ± 1.1	3.96 ± 25.0	$t = 1.39$ $p = 0.192$	3.08 ± 0.5	3.36 ± 0.6	− 9.91 ± 12.3	$t = -2.72$ $p = 0.020$
Substantia nigra	1.55 ± 0.8	1.24 ± 0.6	16.91 ± 36.5	$t = 1.94$ $p = 0.078$	1.51 ± 0.4	1.71 ± 0.4	− 17.15 ± 30.1	$t = -1.64$ $p = 0.129$

Abbreviations: AST, associative striatum; BP_{ND}, non-displaceable binding; CHR, clinical high; CHR-CU, cannabis-using CHR; GP, globus pallidus; MIST, Montreal Imaging Stress Task; SMCT, Sensorimotor Control Task.
BP_{ND} data is consistent with % change data.

with a history of drug use other than cannabis were excluded from the CHR-CU group, differences in % change remained significant in the whole striatum ($F = 7.415$, $df = 1,14$, $p = 0.016$), AST ($F = 8.867$, $df = 1,14$, $p = 0.010$), and LST ($F = 5.157$, $df = 1,14$, $p = 0.039$), but not in the SMST ($F = 3.46$, $df = 1,14$, $p = 0.084$), GP ($F = 0.697$, $df = 1,14$, $p = 0.418$), and SN ($F = 1.723$, $df = 1,14$, $p = 0.210$).

Negative SOPS scores were significantly inversely correlated with % change in BP_{ND} in the CHR group (Figure 2; LST ($r = -0.66$, $p = 0.020$), GP ($r = -0.72$, $p = 0.009$), and SN ($r = -0.89$, $p < 0.001$), but not in the AST ($r = -0.39$, $p = 0.214$), SMST ($r = 0.37$, $p = 0.236$), or the striatum taken as a whole ($r = -0.10$, $p = 0.762$) in CHR subjects with higher scores in negative SOPS symptoms exhibiting lower %change in [^{11}C]-PHNO BP_{ND}, with no correlation in the CHR-CU group ($r = 0.001$, $p = 0.422$). Correlations between changes in positive SOPS and BP_{ND} values are presented in the Supplementary Materials. No additional significant correlation was observed between positive, disorganization and general SOPS symptoms, and %change in any of the regions studied.

We used a voxel-wise analysis to test without *a priori* anatomical hypotheses (ie, ROI definition) whether we could find a difference in SMCT [^{11}C]-(+)-PHNO binding between groups and to confirm the increased tracer binding following the stress task in CHR-CU. In line with the ROI outcome, we found no difference (no clusters with $p > 0.05$) between groups when comparing SMCT scans between groups. Clusters of robust stress-induced increase in BP_{ND} at the level of the dorsal striatum were detected in CHR-CU when comparing MIST to SMCT scan (Figure 3). In contrast, no significant clusters of increased BP_{ND} were detected in CHR (Figure 3).

Changes in salivary cortisol AUC were not significantly different between groups ($F = 1.37$, $df = 1,20$, $p = 0.256$). However, the percent change in the cortisol response between the control task and stress task was significantly positively associated with the stress-induced change in [^{11}C]-(+)-PHNO BP_{ND} in the AST ($r = 0.68$, $p = 0.032$), SMST ($r = 0.67$, $p = 0.036$), and the whole striatum ($r = 0.66$, $p = 0.039$) in CHR (Mizrahi *et al*, 2012) but not in CHR-CU.

There was a trend-level relationship between age of onset of cannabis use and AST %change in [^{11}C]-(+)-PHNO BP_{ND} ($r = 0.56$, $p = 0.09$) such that a greater increase in tracer BP_{ND} was associated with earlier age of onset; however, there was no significant association with cannabis lifetime use or years used for any brain region ($p > 0.102$). Exploring the behavioural aspects of cannabis use (as assessed with the MCQ), we observed an increase in the emotionality subscale such that CHR-CU were anticipating a relief from negative mood on the day of the stress scan but not on the day of the control scan ($F = 4.68$, $p = 0.04$). Interestingly, we observed that among the CHR-CU subjects, lower [^{11}C]-(+)-PHNO BP_{ND} in the AST, LST, SMST, and whole striatum in the SMCT condition correlated with higher emotionality ($r = 0.74$, $p = 0.009$; $r = 0.75$, $p = 0.008$; $r = 0.60$, $p = 0.05$; and $r = 0.76$, $p = 0.007$, respectively) and expectancy (SMST: $r = 0.62$, $p = 0.04$ and whole striatum: $r = 0.62$, $p = 0.04$, respectively) following the MIST. In addition, CHR-CU subjects with higher emotionality scores preceding the MIST scan exhibited larger %change in [^{11}C]-(+)-PHNO BP_{ND} in the LST ($r = 0.64$, $p = 0.035$). MCQ measurements did not correlate with the cortisol measurements.

PET scans took place on average 11 ± 5.3 (3.5–18.5 range) and 13.3 ± 5.8 (2.5–20 range) h (SMCT and MIST, respectively) since the last cannabis use ($F = 0.91$, $p = 0.351$), with 4 of 12 CHR-CU subjects reported using cannabis < 10 h before the SMCT or MIST scan (Table 1). The PET imaging results presented in the study (increased stress-induced BP_{ND} in the CHR-CU, no significant difference in SMCT BP_{ND}) were no different when these four subjects are excluded from the analyses. In addition, no correlation was observed between the hours since last cannabis use before the scan and the PET outcomes (BP_{ND} of the SMCT or MIST session, % change in BP_{ND}).

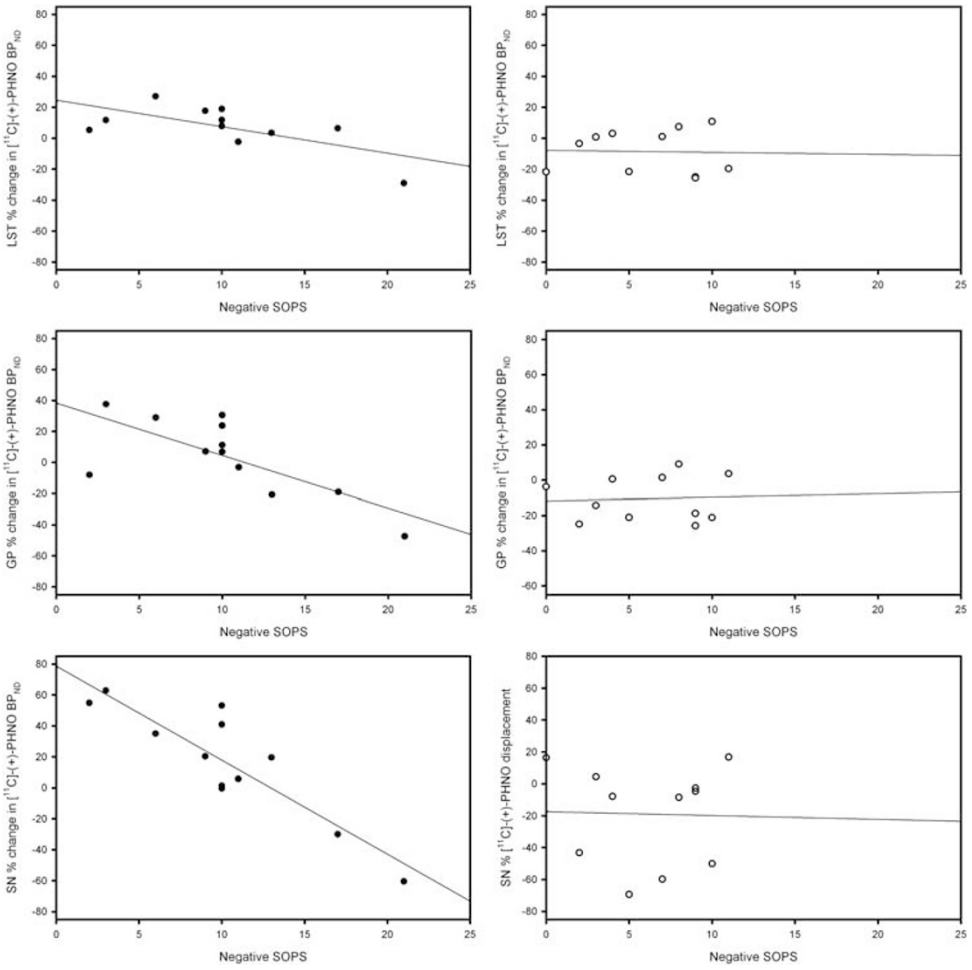

Figure 2 Correlation between the % change of [^{11}C]-(+)-PHNO non-displaceable binding (BP_{ND}) with negative Scale of Prodromal Symptoms (SOPS) in the limbic striatum (LST, top row), globus pallidus (GP, middle row), and substantia nigra (SN, bottom row) of clinical high risk (CHR, left column, solid circles) and cannabis-using CHR (CHR-CU) subjects (right column, empty circles).

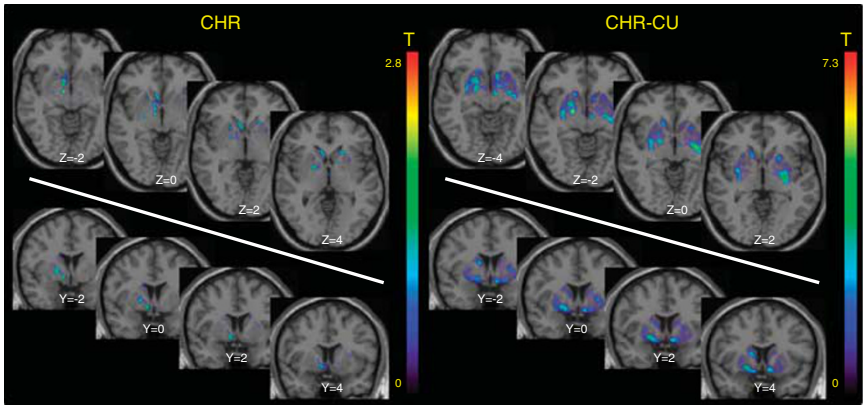

Figure 3 *t*-Statistical map overlaid on magnetic resonance imaging (MRI) template (International Consortium for Brain Mapping template) illustrating clusters of significant increase in [^{11}C]-(+)-PHNO binding (non-displaceable binding (BP_{ND})) in response to the stress scan in clinical high risk (CHR, left) and CHR with concurrent cannabis use (CHR-CU, right) (MNI coordinates: −26–10 2; $t_{max} = 6.42$, cluster size = 495, *p*-uncorrected < 0.001, *p*-corrected < 0.002 and 22 6–6; $t_{max} = 5.17$, cluster size = 395, *p*-uncorrected < 0.001, *p*-corrected < 0.007).

DISCUSSION

Our results suggest increased stress-induced [^{11}C]-(+)-PHNO binding in the striatum and all its functional subdivisions (AST, LST, and SMST), as well as in the SN in CHR cannabis-dependent individuals, despite increased positive attenuated psychotic symptoms in response to stress. Our findings using ROI approach are corroborated by the voxel-wise analysis.

Low striatal DA receptor ($D_{2/3}$) availability and low amphetamine-induced DA release in the ventral striatum have been observed with substance-use disorders, including alcoholism (Martinez *et al*, 2005; Volkow *et al*, 1996), heroin (Martinez *et al*, 2012), cocaine (Volkow *et al*, 1993), and methamphetamine (Volkow *et al*, 2001) abuse. Conversely, studies in patients with schizophrenia and those in putative prodromal states have shown a sensitization of dopaminergic neurotransmission, manifested as increased DA release (ie, reduced tracer binding) in response to amphetamine (Abi-Dargham *et al*, 1998; Laruelle *et al*, 1999; Laruelle *et al*, 1996) or stress (Mizrahi *et al*, 2012), compared with healthy volunteers. Importantly, a recent study in patients with schizophrenia and substance dependence (including cannabis) showed a blunted DA response to amphetamine (Thompson *et al*, 2013), consistent with our results. Observed changes in stress-induced alterations in BP_{ND} in CHR-CU do not significantly differ from past observations in healthy volunteers (Mizrahi *et al*, 2012), although a clear trend is present where the CHR-CU group exhibits a general increase in BP_{ND} (hence, negative [^{11}C]-(+)-PHNO binding %change) in response to stress (AST: HV = -2.87 ± 9.21, CHR-CU = -7.04 ± 7.46, F = 1.48, df = 1,22, $p = 0.236$; LST: HV = -1.69 ± 13.44, CHR-CU = -10.48 ± 14.99, F = 2.29, df = 1,22, $p = 0.145$; SMST: HV = -1.35 ± 9.45, CHR-CU = -7.01 ± 7.82, F = 2.55, df = 1,22, $p = 0.125$; and whole striatum: HV = -2.41 ± 9.10, CHR-CU = -7.35 ± 7.63, F = 2.08, df = 1,22, $p = 0.162$).

A major difference between the agonist [^{11}C]-(+)-PHNO and the commonly used antagonist radiotracer [^{11}C]-raclopride is its ~20-fold higher affinity for the D_3 receptors over the D_2 (Narendran *et al*, 2006; Rabiner *et al*, 2009), resulting in increased sensitivity to DA levels in D_3-rich regions. The D_3 proportion of total DA receptor density varies from 100 and 67% in D_3-rich regions (SN and GP, respectively; Searle *et al*, 2010; Tziortzi *et al*, 2011) to 10–40% in other striatal regions (Searle *et al*, 2010; Searle *et al*, 2013; Tziortzi *et al*, 2011). Recent studies have reported increased D_3 receptor availability in addictions by showing increased [^{11}C]-(+)-PHNO BP_{ND} in D_3-rich areas of chronic methamphetamine users (Boileau *et al*, 2012) and elevated binding of the ^{3}H-labeled version of (+)-PHNO in rats following prolonged THC exposure (Ginovart *et al*, 2012). In our current work we did not observe any differences in the control task BP_{ND} values between CHR and CHR-CU (albeit our baseline is not a 'true' baseline). The stress-induced [^{11}C]-(+)-PHNO BP_{ND} increase, however, is observed in both relatively D_2-rich (striatum) and D_3-rich (SN) regions of the brain. Although recent work in addiction research has been oriented towards the D_3 DA receptor subtype, the differences in stress-induced %change in BP_{ND} observed in our study were more significant in the D_2-rich regions. Taking into consideration that no stress-induced [^{11}C]-(+)-PHNO BP_{ND} change was observed in healthy cannabis users (Mizrahi *et al*, 2013), our results suggest that cannabis dependence presents with a reversal of the dopaminergic sensitization in D_2-rich regions of CHR subjects (Mizrahi *et al*, 2012).

The observed increase in the MCQ emotionality subscale at the day of the stress task (compared with the SMCT) confirms the link between stress and cannabis craving, suggesting that a stressful experience is associated with increased anticipation of relief from the negative mood. Linkage between lower receptor availability during the control task, reflective of either receptor downregulation or higher DA levels, and higher emotionality and expectancy indices may reflect the putative relationship between the dopaminergic system and craving (Volkow *et al*, 2006). Although our study is not powered to evaluate this further, the finding supports future investigations exploring the relationship between dopaminergic signaling in addictions and drug craving under stress. Considering that D_3 receptors may be involved in the regulation of motivation and reward (Murray *et al*, 1994), [^{11}C]-(+)-PHNO could have a major role in future efforts.

Reduced total scores in the negative dimension of SOPS observed in CHR-CU compared with CHR support previous reports of decreased negative symptoms among cannabis-using patients with schizophrenia (Addington and Addington, 1998; Bersani *et al*, 2002; Compton *et al*, 2004; Dubertret *et al*, 2006; Peralta and Cuesta, 1992). The patients with cannabis use may represent a higher-functioning subgroup of CHR (DeRosse *et al*, 2010), who present with better social skills needed to purchase drugs. Alternatively, cannabis use may alleviate the negative symptoms in CHR subjects. High rate of cannabis use among patients with schizophrenia and those at risk of developing the disease could therefore be consistent with the addiction vulnerability hypothesis (Chambers *et al*, 2001). Alternatively, a putative mechanism underlying the self-medication perspective comes from recent animal studies that have implicated the endogenous cannabinoid anandamide through its action on the brain CB1 receptors in the regulation of the hypothalamus–pituitary–adrenal (HPA) axis of stress response (Hill *et al*, 2009; Hill *et al*, 2005; Rademacher *et al*, 2008). It is conceivable that stimulation of CB1 receptors by exogenous cannabis suppresses the HPA response, attenuating the stress-induced DA release. Future longitudinal studies will be able to address this issue. In our study, difference in cortisol response between the control and stress conditions in CHR correlated with stress-induced striatal BP_{ND} changes, but not in the CHR-CU group, warranting further studies into possible decoupling of HPA response from dopaminergic signaling in cannabis users.

Some limitations are typical in neurochemical brain-imaging studies. First, we did not control for type of marijuana used by subjects. Over the past decades, the THC content in marijuana consumed in North America has been increasing because of the availability of more potent strains. This may have significantly affected the results of the present study, given that the different components of the marijuana used may have opposite effects on brain DA function (Murphy *et al*, 1990); however, all study participants were recruited from the same geographical area and within the same time frame. Second, the inclusion in the CHR-CU subjects who have a past occasional use of drugs other than cannabis reflects the nature of the population from which the subjects were recruited. The use of illegal substances, as well as cigarette smoking, were suggested to alter striatal dopaminergic signaling. CHR population exhibits high rates of substance and tobacco use (Rosen *et al*, 2006), making exclusion of any past use or smokers impractical. However, present results (significant difference

in stress-induced %change in BP_{ND} between CHR and CHR-CU groups) remain even after removing subjects that used tobacco or had a history of use of other drugs from the analysis. Third, the period of abstinence from cannabis use varied in the scanned subjects, as abstinence was not among the inclusion criteria. To exclude the possibility of acute effects of cannabis (considered to be modest or less; Bossong *et al*, 2009; Stokes *et al*, 2012), we re-analyzed the data excluding subjects who used cannabis <10 h before the scan, with the presented results remaining significant. In addition, because of the group sizes and the fact that our study was powered to test a difference on [^{11}C]-(+)-PHNO binding between groups, the correlations we explored with clinical measures are not significant when correcting for multiple comparisons using the Bonferroni approach, except for the correlation between whole striatum %change in [^{11}C]-(+)-PHNO BP_{ND} and emotionality index in CHR-CU subjects, and the total SOPS negative symptoms and %change in [^{11}C]-(+)-PHNO BP_{ND} in the SN. In addition, it has recently been suggested that [^{11}C]-(+)-PHNO may not be at tracer dose in the D_3-rich regions such as the SN, which would hinder accurate quantification (Gallezot *et al*, 2009; Rabiner and Laruelle, 2010; Searle *et al*, 2013). Incorporation of the factor of injected mass in the comparison of %change of tracer BP_{ND} did reduce the difference observed in SN, but not in the D_2-rich regions. Importantly, our findings are present in all brain regions investigated, including D_2-specific regions such as the AST where this effect is not present (Shotbolt *et al*, 2011). To exclude any potential effect of the putative specific [^{11}C]-(+)-PHNO binding in the cerebellum, we have compared cerebellar tracer uptake between SMCT and MIST tasks, showing almost complete overlap (Supplementary Materials). Although the cerebellum standard uptake values over time show CHR-CU tracer uptake during MIST scan to be slightly lower than the SMCT scan, the difference is unlikely to explain the findings of the study. Finally, independent confirmation of the PET findings using the MIST procedure in large cohorts are warranted to strengthen the general applicability of the [^{11}C]-PHNO MIST data. Finally, it should be noted that owing to the number of analyses performed, correlations presented in the Supplementary Materials should be considered exploratory data.

In conclusion, our current work presents evidence of stress-induced increased [^{11}C]-(+)-PHNO binding in CHR subjects who concurrently use cannabis compared with non-cannabis-using CHR, supporting recent publications exploring amphetamine DA response in patients with schizophrenia and substance dependence (Thompson *et al*, 2012), and cannabis users who have psychotic experiences (Bloomfield *et al*, 2013). Our findings highlight the interaction between stress, dopaminergic signaling, and cannabis, opening new venues for future research. Given that drug use is highly dependent on the environment, and recent epidemiological data showing how environmental risk factors affect brain function (Lederbogen *et al*, 2011), further studies exploring the neurochemical changes of the interaction between stress, cannabis use, and schizophrenia are warranted.

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The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)