Schizophrenia Bulletin vol. 44 no. 3 pp. 542–551, 2018
doi:10.1093/schbul/sbx042
Advance Access publication June 13, 2017

Nigral Stress-Induced Dopamine Release in Clinical High Risk and Antipsychotic-Naïve Schizophrenia

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Background: **Striatal dopamine (DA) synthesis capacity and release are elevated in schizophrenia (SCZ) and its putative prodrome, the clinical high risk (CHR) state. Striatal DA function results from the activity of midbrain DA neurons projecting mainly from the substantia nigra (SN). Elevated stress-induced DA release in SCZ and CHR was observed in the striatum; however, whether it is also elevated in the SN is unclear. The current study aims to determine whether nigral DA release in response to a validated stress task is altered in CHR and in antipsychotic-naïve SCZ. Further, we explore how DA release in the SN and striatum might be related.** ***Methods:*** **24 CHR subjects, 9 antipsychotic-naïve SCZ and 25 healthy volunteers (HV) underwent 2 positron emission tomography (PET) scans using the DA $D_{2/3}$ agonist radiotracer, [^{11}C]-(+)-PHNO, which allows simultaneous investigations of DA in the SN and striatum. Psychosocial stress-induced DA release was estimated as the percentage differences in BP_{ND} (%[^{11}C]-(+)-PHNO displacement) between stress and sensory-motor control sessions.** ***Results:*** **We observed a significant diagnostic group by session interaction, such that SCZ exhibited greater stress-induced [^{11}C]-(+)-PHNO % displacement (25.90% ± 32.2%; mean ± SD), as compared to HVs (−10.94% ± 27.1%). Displacement in CHRs (−1.13% ± 32.2%) did not differ significantly from either HV or SCZ.** ***Conclusion:*** **Our findings suggest that elevated nigral DA responsiveness to stress is observed in antipsychotic-naïve SCZ.**

Key words: dopamine/substantia nigra/psychosis/positron emission tomography/clinical high risk/striatum

Introduction

Elevations in striatal dopamine (DA) synthesis capacity and release in response to a challenge (DA drugs or stress) are key features in schizophrenia (SCZ).[1–3] Increased striatal DA synthesis capacity[4,5] and release[3,6] are also observed in individuals at clinical high risk (CHR) for psychosis, suggesting a pre-morbid altered neurochemical state. CHR is a well-defined cohort with attenuated psychotic symptoms and/or genetic risks and recent functional deterioration[7] with a conversion rate 25%–35% over 24–36 months.[8,9]

The observation of increased presynaptic DA synthesis and release in SCZ and CHR, raises the question of upstream functional changes of DA transmission. Striatum receives dopaminergic innervations from the midbrain, particularly from substantia nigra (SN) and ventral tegmental area (VTA). Evidence indicates that alterations in striatal DA function are particularly apparent in the associative subdivision of the striatum (AST),[3,4,10] which predominantly receives projections from the pars compacta of SN (SNc).[11–13] Thus, the increased AST DA synthesis and release in psychosis may reflect an increased dopaminergic function in the SN.

Increased nigral DA activity in SCZ is supported by both postmortem and in vivo evidence. Postmortem studies have reported elevated tyrosine hydroxylase activity[14–16] and D_2 receptor levels[17] in the SN of SCZ compared to healthy volunteers (HV; but see[18,19]). Similarly, in vivo studies reported higher midbrain DA turnover (estimated by k_{loss} of [^{18}F]fluorodopamine)[20] and DA synthesis capacity (measured by K_i^{cer} of [^{18}F]-DOPA)[14] in SCZ compared to HV. In vivo D_2 receptor availability has been reported to be elevated in antipsychotic-free/naïve SCZ by one,[21]

but not all studies,[22] relative to HV. Investigations using functional magnetic resonance imaging (fMRI) have observed elevated SN activity in SCZ during cognitive tasks[23,24] and during presentation of motivationally neutral stimuli.[25] A functional relationship of DA activity in SN and striatum may be relevant to clinical presentation as greater psychotic symptoms were associated with not only higher midbrain DA synthesis capacity[14] but also greater SN-caudate functional connectivity.[23] However, it is not known if, or under what conditions, alterations of DA function in striatum may relate to midbrain DA function. Whereas striatal DA synthesis capacity[26] and release is apparently blunted in otherwise healthy cannabis users, midbrain DA release has surprisingly been reported to be elevated following a challenge with methylphenidate[27] but not with amphetamine[28] or stress.[29]

Despite evidence for clinically relevant changes in the SN DA system in SCZ, few studies have explored nigral DA release in humans, fewer still in CHR. Studies using [^{11}C]-(+)-PHNO have investigated nigral DA release in response to amphetamine[30,31] and in response to nicotine and a psychosocial stress[29] in nonpsychotic participants. In vivo imaging evidence of a functional relationship between SN and striatum DA in healthy humans comes from a study that observed a negative association between baseline SN/VTA $D_{2/3}$ receptor availability and amphetamine-induced DA release in striatum,[32] suggesting a measurable contribution of SN/VTA autoreceptors in modulating downstream DA release. To date, only 1 study has examined nigral DA release in SCZ. Using the high-affinity $D_{2/3}$ ligand [^{11}C]-FLB-457, Slifstein et al[33] reported decreased DA release in extra-striatal regions in response to an amphetamine challenge in SCZ, including a trend-level decrease in the midbrain (SN/VTA). However, the relationship between DA release capacity in the SN and in striatum could not be explored in that [^{11}C]-FLB-457 study.

[^{11}C]-(+)-PHNO is a DA $D_{2/3}$ agonist ligand that permits a simultaneous investigation of DA release in the SN and striatum. The overall regional signal of [^{11}C]-(+)-PHNO is a function of its differential affinities for D_3 vs D_2 receptors and the availability of each receptor subtype in a given region. In SN, the majority of the [^{11}C]-(+)-PHNO signal is attributable to binding to D_3 receptors (83%–100%), whereas in dorsal striatum D_2 binding predominates (D_3 fraction: 0%–26%).[34] Like D_2 receptors, somatodendritic DA D_3 receptors expressed by midbrain DA neurons[35,36] are thought to act as autoreceptors.[37,38] As such, changes in [^{11}C]-(+)-PHNO binding potential in the SN reflect changes in the concentration of extracellular DA, which acts on $D_{2/3}$ autoreceptors to modulate SN/VTA DA neuron activity[39–41] and, in turn, striatal DA release.[42]

Based on postmortem and in vivo evidence of increased DA synthesis and turnover rate in the SN,[14–16] but see Slifstein et al,[33] we postulated an increased nigral DA release in antipsychotic-naïve SCZ and individuals at CHR for psychosis in response to a psychosocial stress paradigm. We also explored the association between changes in [^{11}C]-(+)-PHNO binding in SN and striatum, as well as the association between changes in SN [^{11}C]-(+)-PHNO binding and psychotic symptoms in SCZ and attenuated psychotic symptoms in CHR.

Methods and Materials

Participants

This study combines and extends a cohort described in prior studies,[3,6,29,43] comprising a total 24 CHR, 9 SCZ, and 25 matched HV. This report omits the data of 1 SCZ individual included in an earlier analysis (examining striatum)[3] as we were unable to obtain a reliable estimate of [^{11}C]-(+)-PHNO binding in the SN in both scans. Data for HV and CHR in striatum and SN have been reported previously and are presented here for comparison with SCZ SN data and to explore regional associations in [^{11}C]-(+)-PHNO displacement. As described previously,[3] CHR participants met criteria for prodromal syndromes based on the Structured Interview for Prodromal Syndromes (SIPS),[44] and the SCZ participants met the diagnosis of SCZ or schizophreniform disorder. All CHR and SCZ patients were free of other axis I disorders except 3 CHR individuals and 1 SCZ patient (table 1). All patients were antipsychotic-naïve. All participants screened negative for drugs of abuse on urine drug screens conducted at enrollment and on positron emission tomography (PET) scan days, excepting identified cannabis-users, all of whom screened positive for cannabis (table 1). In the cannabis user groups, all HV cannabis users, 11 of 12 CHR cannabis users and of 4 SCZ cannabis users 2 met criteria for cannabis dependence and 2 were in partial remission at the time of the study. All subjects provided written, informed consent to participate.

Assessments

Psychopathology Measures. As described previously,[3] symptom severity in CHR individuals was assessed using the Scale of Prodromal Symptoms (SOPS), a part of the SIPS with excellent inter-rater reliability.[44] In patients with SCZ, psychosis symptoms were assessed using the Positive and Negative Syndrome Scale (PANSS). In addition, all subjects were screened for any axis I psychopathology using the Structured Clinical Interview for DSM-IV conducted by a qualified psychiatrist (R.M.).

Montreal Imaging Stress Task. The Montreal Imaging Stress Task (MIST) which has been validated in fMRI and PET studies,[3,6,45–48] was used to induce psychological stress as described previously.[3] Briefly, subjects performed a series of mental arithmetic tasks on a computer screen that displayed information about the total number

Table 1. Demographic and Clinical Variables

		HV (n = 25)	CHR (n = 24)	SCZ (n = 9)
Age (y)		25.12 (4.45)	23.63 (4.67)	24.11 (5.33)
Education (y)		14.48 (2.00)	13.67 (2.55)	13.63 (2.07)
Ethnicity 1/2/3/4/5		17/0/4/3/1	13/0/7/3/1	5/0/3/0/1
Sex	Male	13	13	6
	Female	12	11	3
Tobacco smoking status	Nonsmoker	21	19	6
	Smoker	4	5	3
Cannabis (current)	Nonuser	12	12	5
	User	13	12	4
Cocaine (history)	Nonuser	23	18	9
	User	2	6	0
Amphetamine (history)	Nonuser	25	22	9
	User	0	2	0
Ecstasy (history)	Nonuser	21	18	9
	User	4	6	0
Clinical Information and PET parameters		HV (n = 25)	CHR (n = 24)	SCZ (n = 9)
SOPS-P		—	11.91 (2.54)[b]	—
SOPS-N		—	8.26 (4.91)[b]	—
PANSS-P		—	—	19.33 (3.84)
PANSS-N		—	—	16.78 (4.12)
Other diagnosis	Anxiety disorder	0	0	1
	Others[a]	0	3	0
Medications used	Anti-depressant	0	1	1
	Others	0	2	0
Amount injected (SD)	Control task	9.50 (1.36)	9.57 (1.73)	9.12 (1.47)
	Stress task	9.93 (0.73)	9.79 (0.96)	9.76 (1.54)
Specific activity (SD)	Control task	1161.94 (488.57)	1107.33 (504.07)	1015.02 (550.59)
	Stress task	1239.25 (443.04)	1243.93 (595.66)	1012.19 (290.99)
Mass injected (SD)	Control task	2.16 (0.67)	2.42 (0.93)	2.54 (0.87)
	Stress task	2.11 (0.67)	2.42 (1.02)	2.65 (0.67)

Note: HV, healthy volunteer; CHR, clinical high risk; SCZ, schizophrenia; Ethnicity (self-reported): 1: White; 2: Mixed/Multiple ethnic groups; 3: Asian/Asian Canadian; 4: Black/African/Caribbean/Black Canadian; 5: Other; PANSS-P and PANSS-N: Positive and Negative Syndrome Scale, positive subscale and negative subscale; SOPS-P and SOPS-N: Scale of Prodromal Symptoms, positive scale and negative scale.
[a]Other diagnoses includes: Major depression in remission (2), alcohol abuse (1).
[b]The SOPS score of 1 CHR participant was not available.

of errors, expected average number of errors, time spent on the current problem, and performance feedback for each problem (correct, incorrect, or timeout). The stress condition of the MIST includes a time constraint that varies according to each individual's performance producing only 20%–30% correct answers. In addition, subjects received negative verbal feedback from the same investigator (R.M.) following a script to ensure verbatim language was delivered to all subjects. The investigator providing feedback was aware of the group membership of participants. Prior to the stress-task, all subjects performed a Sensory Motor Control Task (SMCT) PET session (non-stress) with a similar arithmetic task but without time constraints or negative verbal feedback.

[11C]-(+)-PHNO PET Image Acquisition and Analyses. All 58 subjects completed 2 PET scans (n = 116 PET scans), conducted about a week apart, roughly at the same time of the day. The procedures were described in greater detail previously.[3] Briefly, the SMCT (control task) was performed during the first scan and the MIST (stress task) was performed during the second scan. Stress-induced DA release was quantified as [^{11}C]-(+)-PHNO % displacement = (BP_{ND} SMCT - BP_{ND} MIST) / BP_{ND} SMCT) * 100%.

All scans were carried out using a high-resolution PET CT, Siemens-Biograph HiRez XVI (Siemens Molecular Imaging). Each subject was administered ~333–370 MBq of [^{11}C]-(+)-PHNO and scanned for 90 minutes. A CT transmission scan was acquired for attenuation correction.

PET images were reconstructed with a 2D filtered back-projection algorithm with a ramp filter at Nyquist cut-off frequency and rebinned into 31 time frames (comprising the background frame, followed by [^{11}C]-(+)-PHNO injection and fifteen 60-second frames followed by fifteen 300-second frames).[49] Images and Time Activity Curves (TACs) were systematically inspected visually for head movement, and corrected when motion was visible (33

out of 116 scans, including 10 HV: 15 scans, 10 CHR: 16 scans, and SCZ: 2 scans) using our standard motion-correction technique which relies on frame-to-frame realignment. Specifically, the method involves registration of the no-attenuation-corrected frames using the Automatic Image Registration (AIR) algorithm. The primary region of interest (ROI) was SN; the whole striatum and its functional subdivisions, including AST, limbic (LST), and sensorimotor striatum (SMST)[50,51] were included for comparisons with our previous findings. The analyses were performed in each subject's native PET image space. Time activity curves (TACs) from the SN and the other ROIs were obtained from the dynamic [^{11}C]-(+)-PHNO PET images. [^{11}C]-(+)-PHNO binding potential relative to Non-Displaceable compartment (BP_{ND})[52] was derived using the Simplified Reference Tissue Model[53] with cerebellar cortex as the reference region (cerebellar vermis excluded) as previously described for [^{11}C]-(+)-PHNO analyses.[54,55] Voxel-wise images were generated using the Receptor Parametric Mapping (RPM) software,[56] and spatially normalized to MNI template space using Statistical Parametric Mapping (SPM). A paired *t* test was performed in the MNI template space to compare BP_{ND} between SMCT vs MIST within each group (HV, CHR, and SCZ) at the voxel level. An explicit anatomical mask of the midbrain was applied to define the region of interest. Midbrain gray matter voxels extending from planes $z = -4$ to $z = -14$ on 6 consecutive transverse slices in stereotaxic space (2 mm, MNI space).[30,57] False-discovery rate correction was used as implemented in SPM8 (www.fil.ion.ucl.ac.uk/spm).

Statistical Analysis. All statistical analyses were performed in SPSS version 22.0 (IBM). Demographic measures were compared using 1-way ANOVA for continuous variables, or chi-square tests for categorical variables. Repeated-measures ANCOVA (RM-ANCOVA) was used to test the effects of psychosocial stress on the outcome measure [^{11}C]-(+)-PHNO BP_{ND}, with clinical group as between-subject variable, session (SMCT, MIST) as within-subject variable, and concurrent cannabis use included as a covariate. A significant clinical group × session interaction indicated the group differences of stress-induced change in [^{11}C]-(+)-PHNO binding in the SN, as exemplified by [^{11}C]-(+)-PHNO % displacement. Effect sizes were calculated as partial η^2. To elucidate the role of cannabis use in stress-induced changes in nigral [^{11}C]-(+)-PHNO binding, a supplementary RM-ANCOVA was performed, with cannabis use as between-subject variable, session as within-subject variable, and clinical group allocation as a covariate.

Partial correlation of the 2 measures, (1) BP_{ND} during the SMCT session and (2) stress-induced DA release ([^{11}C]-(+)-PHNO % displacement), were examined between SN and other striatal regions, covarying for cannabis use. Exploratory partial correlations between these 2 measures and clinical symptoms were also examined, covarying for cannabis use. Statistical significance was defined as $P < .05$ unless otherwise stated. Bonferroni correction was used for multiple comparisons for striatal subdivisions (AST, SMST and LST; adjusted *P* value threshold = .017).

Results

Demographic and Clinical Variables

All 3 groups were comparable in demographics (table 1). The stress task was effective in producing subject-tailored failure and elicited subjective stress responses. During the stress task, all subjects, controlling for group allocation and cannabis use, exhibited higher overall perceived stress relative to the control task ($F = 133.419$, $df = 1,104$, $P < .0001$). During the stress task participants reported being less calm ($F = 47.855$, $df = 1,104$, $P < .0001$), satisfied ($F = 98.067$, $df = 1,104$, $P < .0001$), relaxed ($F = 34.893$, $df = 1,104$, $P < .0001$), and pleasant ($F = 30.147$, $df = 1,104$, $P < .0001$) but more strained ($F = 47.854$, $df = 1,104$, $P < .0001$), tense ($F = 54.899$, $df = 1,104$, $P < .0001$), upset ($F = 131.499$, $df = 1,104$, $P < .0001$), and confused ($F = 41.964$, $df = 1,104$, $P < .0001$) compared to the control session. Participants in all groups performed significantly worse during the stress task (number of errors 38.62, 34.96, and 32.00 for HV, CHR, and SCZ, respectively) relative to the control task ($F = 277.212$, $P < .0001$).

[^{11}C]-(+)-PHNO Binding (BP_{ND}) Across Control and Stress Sessions

The main effect of group on nigral BP_{ND} was not significant ($F = 0.269$, $df = 2,54$, $P = .765$), but the session effect was significant ($F = 12.396$, $df = 1,54$, $P = .001$), with lower nigral BP_{ND} in the stress session than in the control session. As hypothesized, we observed a significant group × session interaction in the SN ($F = 6.896$, $df = 2,54$, $P = .002$), such that the % [^{11}C]-(+)-PHNO displacement was −10.94% ± 27.1% (mean ± SD) in HV, −1.13% ± 32.2% in the CHR, and 25.90% ± 32.2% in SCZ (figure 1). Follow-up paired comparisons (Bonferroni corrected for 3 comparisons, adjusted α-level = .017) confirmed a significant group × session interaction between HV and SCZ ($F = 15.250$, $df = 1,31$, $P < .001$), with greater displacement in SCZ; and a trend towards a significant group × session interaction between CHR and SCZ ($F = 5.093$, $df = 1,30$, $P = .031$), with greater displacement in SCZ. The group × session interaction between HV and CHR was not significant. Similar group × session interactions were observed across the striatum ($F = 4.362$, $df = 2,54$, $P = .018$), where SCZ had higher stress-induced [^{11}C]-(+)-PHNO displacement. The group × session interaction remained significant after controlling for tobacco (SN: $F = 6.487$, $P = .003$; striatum: $F = 5.01$, $P = .01$), or

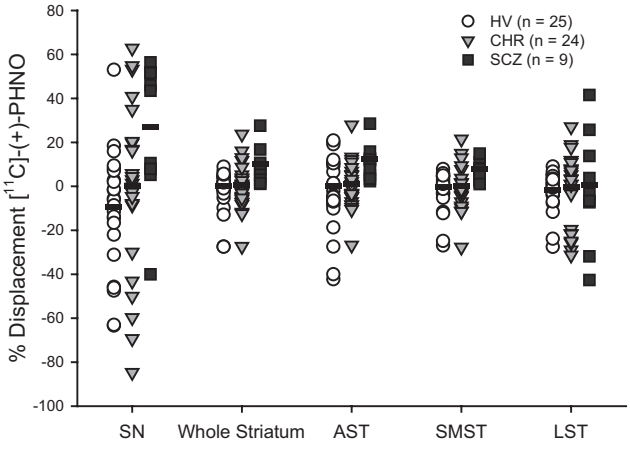

Fig. 1. Significant effect of clinical group on stress-induced [^{11}C]-(+)-PHNO displacement in the substantia nigra (SN) (F = 6.896, df = 2,54, P = .002) and the whole striatum (F = 4.362, df = 2,54, P = .018), including associative striatum (AST) (F = 6.131, df = 1,54, P = .004), sensorimotor striatum (SMST) (F = 3.384, df = 2,54, P = .041), but not in the limbic striatum (LST) (F = 0.173, df = 2, 54, P = .842). HV, healthy volunteer; CHR, clinical high risk; SCZ, schizophrenia.

gender (SN: F = 6.484, P = .003; striatum: F = 4.745, P = .0127). Removal of individuals that reported past recreational use of drugs other than cannabis (table 1) did not significantly affect the primary outcome measures: overall group × session interaction in SN (F = 7.275, P = .002) and whole striatum (F = 4.577, P = .015). Post hoc analyses revealed that the effect was driven by the % [^{11}C]-(+)-PHNO displacement in dorsal subregions of the striatum, including the AST (F = 6.131, df = 1,54, P = .004) and SMST (F = 3.384, df = 2,54, P = .041), but not in the ventral LST subregion (F = 0.173, df = 2,54, P = .842).

In the second analysis examining the effect of cannabis use in SN, the main effect of cannabis use on nigral BP_{ND} across sessions was not significant (F = 2.089, P = .154), but the session effect was significant (F = 9.284, P = .004). We observed a significant cannabis use × session interaction (F = 8.785, P = .004), with greater [^{11}C]-(+)-PHNO % displacement in non-cannabis users (10.90%) relative to users (−13.22%) across groups. The effect of cannabis use on [^{11}C]-(+)-PHNO displacement did not differ between diagnostic groups (F = 0.529, P = .592).

Voxel-Based Analyses

In line with the ROI analyses outcome, we observed 2 significant clusters bilaterally in the midbrain with significant decreases in BP_{ND} in the SCZ group suggesting a significant DA release in the stress condition (Montreal Neurological Institute coordinates: 6, −26, 4, t_{max} = 5.01, cluster size = 82, P = .008; and −10, −20, −10; t_{max} = 3.03, cluster size = 53, P = .032, FDR cluster corrected; supplementary figure 1C). Employing the more conservative FWE correction led to similar results: Montreal Neurological Institute coordinates: 6, −26, 4, t_{max} = 5.01, cluster size = 82, P = .009; and −10, −20, −10; t_{max} = 3.03, cluster size = 53, P = .073. No significant differences were observed between the conditions in the HV (supplementary figure 1A) or CHR groups (supplementary figure 1B).

Correlational Analyses of BP_{ND} SMCT and Displacement in SN and Striatum

[^{11}C]-(+)-PHNO BP_{ND} SMCT in the SN and whole striatum were positively correlated across all subjects (r = .519, P < .001). Significant correlations were also observed in all the striatal subdivisions (AST: r = .511, P < .001; SMST: r = .429, P = .001; LST: r = .471, P < .001, with adjusted threshold P < .017). In the subgroup analyses, correlations between SN SMCT BP_{ND} and the striatal subregions were observed only in the CHR group (Whole striatum: r = .768, P < .001; AST: r = .735, P < .001; SMST: r = .663, P = .001; LST: r = .726, P < .001, with adjusted threshold P < .017) but not in the SCZ or HV groups (table 2, figure 2A). The significant correlation in CHR was observed primarily in non-cannabis users (r = .895, P < .001) but not in cannabis users (r = .470, P = .123).

[^{11}C]-(+)-PHNO displacement in the SN and whole striatum had a positive association across all subjects (r = .290, P = .029; figure 2B), similar associations were also observed in its dorsal subdivisions (AST: r = .306, P = .021; SMST r = .305, P = .021, with adjusted threshold P < .017) but not in the ventral subdivision (LST: r = .133, P = .326). The significant correlation of [^{11}C]-(+)-PHNO displacement in the SN and in striatum was observed in non-cannabis users (striatum: r = .545, P = .002; AST: r = .543, P = .002; SMST: r = .388, P = .037; LST: r = .388, P = .037) but not in cannabis users (striatum: r = .224, P = .242; AST: r = .324, P = .087; SMST: r = .394, P = .034; LST: r = −.133, P = .493). Investigating diagnostic groups separately, the significant correlation in CHR was observed primarily in non-cannabis users (r = .646, P < .023) but not in cannabis users (r = −.033, P = .920).

Correlational Analyses of BP_{ND} SMCT and Displacement in SN With Clinical Positive and Negative Symptoms

In CHR, [^{11}C]-(+)-PHNO BP_{ND} SMCT in SN was not significantly correlated with SOPS positive (r = .171, P = .446) or negative symptoms score (r = −.292, P = .187). In contrast, [^{11}C]-(+)-PHNO displacement in SN was negatively associated with SOPS negative symptoms score (r = −.616, P = .002, adjusted threshold P < .025 for 2 comparisons, positive and negative symptoms) but not (attenuated) positive symptoms scores (r = .100, P = .658), controlling for cannabis use (figure 2C). The significant correlation was observed

Table 2. Correlations Table for BP_{ND} in the Control Session (BP_{ND} SMCT) and Displacement in SN With Striatum and its Subregion AST, Controlled for Cannabis Use

Correlations of [11C]-(+)-PHNO BP_{ND} SMCT Between SN and Striatum				
	SN BP_{ND} SMCT			
	All Groups	HV	CHR	SCZ
AST BP_{ND} SMCT	r = .511 ***P* < .001**	r = .114 P = .595	r = .735 ***P* < .001**	r = .138 P = .745
Whole striatum BP_{ND} SMCT	r = .519 ***P* < .001**	r = .181 P = .397	r = .768 ***P* < .001**	r = −.071 P = .866
Correlations of [11C]-(+)-PHNO % displacement between SN and striatum				
	SN %displacement			
	All groups	HV	CHR	SCZ
AST %displacement	r = .306 P = .021	r = −.054 P = .801	r = .368 P = .084	r = .064 P = .881
Whole striatum %displacement	r = .290 ***P* = .029**	r = −.010 P = .965	r = .382 P = .072	r = −.131 P = .758

Note: HV, healthy volunteer; CHR, clinical high risk; SCZ, schizophrenia; SN, Substantia nigra; AST, associative striatum; SMCT, Sensory Motor Control Task. Data presented across all groups and within each individual diagnostic group. For striatal subregions, Bonferroni correction was used for multiple comparisons with adjusted P value threshold = .017; P values below the threshold for significance are indicated in BOLD).

primarily in non-cannabis users (r = −.886, P < .001) but not in cannabis users (r = −.028, P = .935).

In SCZ, [11C]-(+)-PHNO BP_{ND} SMCT in SN was not significantly correlated with PANSS positive (r = .540, P = .167) or negative symptoms scores (r = −.351, P = .395). [11C]-(+)-PHNO displacement in SN had trend towards a negative association with PANSS negative symptoms score (r = −.767, P = .026, adjusted threshold P < .025 for 2 comparisons, positive and negative symptoms), controlled for cannabis use, but not positive symptoms (r = .687, P = .060).

Discussion

To our knowledge, this is the first study to investigate stress-induced changes in nigral [11C]-(+)-PHNO binding in antipsychotic-naïve SCZ. We observed a significant diagnostic group by session interaction, such that SCZ exhibited greater stress-induced [11C]-(+)-PHNO % displacement (25.90%), as compared to HVs (−10.94%). Across all subjects, [11C]-(+)-PHNO % displacement in SN was correlated with displacement in the striatum, an effect observed primarily in cannabis nonusers across diagnostic groups, and within non-cannabis-using CHR. Exploratory analyses revealed an association of lower [11C]-(+)-PHNO % displacement in SN with greater negative symptoms in CHR and SCZ.

The present results are in agreement with the findings of increased midbrain DA synthesis capacity[14] and DA turnover[20] in SCZ. However, our results are at odds with a recent report by Slifstein et al[33] of amphetamine-induced [11C]FLB-457 change (P = .10) in midbrain in SCZ. Differences between our studies may have contributed to the divergent observations, including composition of the SCZ cohorts (antipsychotic-naïve vs antipsychotic-free; recent onset vs chronic SCZ), challenge condition (amphetamine vs psychosocial stress), and radioligand (D_3-preferring [11C]-(+)-PHNO vs $D_{2/3}$ ligand [11C]FLB-457). Within the SN the signal from [11C]-(+)-PHNO[34,58] and [11C]FLB-457 are thought to predominantly reflect binding to D_3 and D_2 receptors respectively, which may reflect DA release in histologically distinct subregions such as the D_3-dense rostral pars reticulata (SNr) or D_2-predominant pars compacta (SNc) or the VTA.[59] Further, differences between stimulant and stress-induced DA release in extra-striatal regions have been observed previously, and may reflect the different mechanisms involved in direct pharmacologic action on DA neurons vs endogenous activation of stress-related pathways.[60]

Although we did not observe a significant change in [11C]-(+)-PHNO binding between CHR and HV, one explanation is that following stress, CHR cannabis users and nonusers exhibit changes in [11C]-(+)-PHNO binding that are opposite in direction.[6] In addition, there is some evidence that individual differences including maternal care,[61] personality traits,[62] or repeated exposure to amphetamine[63] may also influence stress-induced DA release in humans. Although the present sample includes individuals with exposure to recreational drugs other than cannabis, removal of these individuals from the analysis

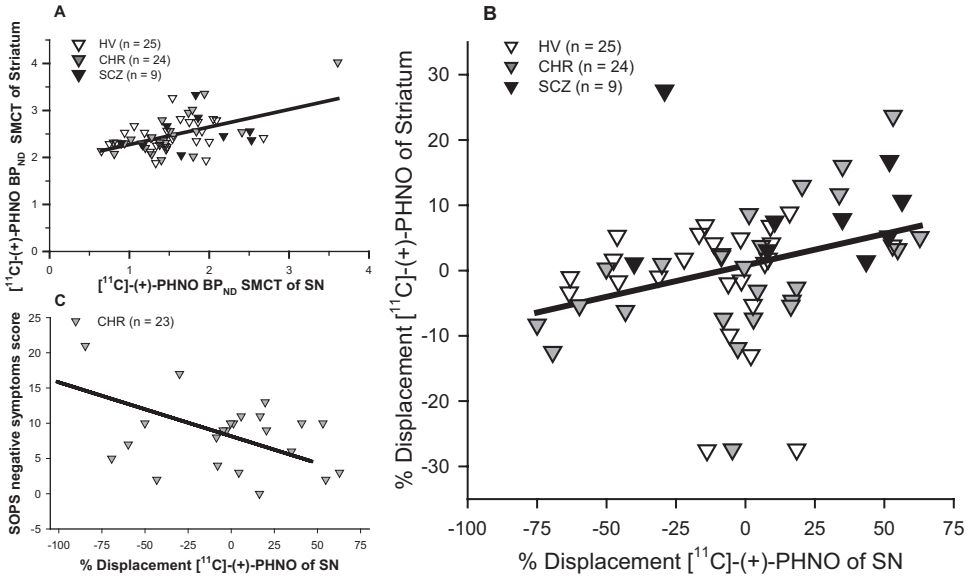

Fig. 2. (A) [^{11}C]-(+)-PHNO BP_{ND} in the control session (SMCT) in striatum and SN. A significant positive association was observed across all groups (r = .519, P < .001); (B) % Displacement of [^{11}C]-(+)-PHNO, quantified as (BP_{ND} SMCT − BP_{ND} MIST) / (BP_{ND} SMCT) * 100%. A positive association of displacement in SN and striatum was observed across all groups (r = .290, P = .029). (C) [^{11}C]-(+)-PHNO displacement in SN and SOPS negative symptoms scores. A significant negative association was observed in CHR (r = −.616, P = .002) (Bonferroni correction was used for multiple comparisons with adjusted P value threshold = .025). Correlations controlled for cannabis use. HV, healthy volunteer; CHR, clinical high risk; SCZ, schizophrenia; MIST, Montreal Imaging Stress Task; SMCT, Sensory Motor Control Task; SN, substantia nigra; SOPS: Scale of Prodromal Symptoms.

did not significantly affect the primary outcome measures. Finally, the lack of significant differences between CHR and HV may be related to the high variability in binding estimates observed in the SN, a relatively small ROI.

Based on the putative modulatory effect of $D_{2/3}$ autoreceptor activation on nigral DA neuron activity,[39–41] in HV we expected a negative association between the stress-induced [^{11}C]-(+)-PHNO binding change in the SN and in the striatum. We did not find such association in HV. The positive association between [^{11}C]-(+)-PHNO displacement in the SN and striatum in non-cannabis-using CHR, but not in HV or SCZ, provides a weak support of an altered balance of DA transmission in the SN and striatum in this putative prodromal stage of psychosis. Excessive nigral DA synthesis[14] and turnover[20] in psychosis might be expected to result in both excessive terminal (striatum) axonal and local (SN) somatodendritic DA release following stress-related activation of DA pathways. Future investigations may require a larger sample or should incorporate a measure of true baseline $D_{2/3}$ receptor availability. Baseline SN $D_{2/3}$ receptor availability has been reported to modulate striatal DA release, however our control condition itself may be expected to recruit DA activity[64] and so does not permit estimation of a true baseline $D_{2/3}$ availability.

In line with previous reports that cannabis users exhibit reduced DA release in striatum,[6,27–29] cannabis users exhibited decreased nigral [^{11}C]-(+)-PHNO displacement relative to nonusers, although in our sample the effect was not significantly associated with recency of cannabis exposure or cannabis use frequency (for details see:[6,29]). Our findings of decreased DA stress responsivity across somatodendritic (SN) and DA terminal fields (AST)[6] in chronic cannabis users suggest that alterations to DA neurotransmission may extend beyond reduced DA synthesis and release capacity in striatum.[26–28]

As previously reported,[6] in CHR cannabis nonusers the magnitude of changes in nigral [^{11}C]-(+)-PHNO binding was negatively associated with attenuated negative but not positive symptoms. Our exploratory observation of a similar association in SCZ provides preliminary support of the result in CHR. These findings are consistent with a recent study in healthy cannabis-dependent subjects where lower DA release in AST was associated with higher ratings of negative symptoms.[28]

The current study has several limitations, many of which are inherent to neurochemical PET studies. The relatively low signal-to-noise ratio in SN produced higher variability of [^{11}C]-(+)-PHNO BP_{ND} and [^{11}C]-(+)-PHNO binding changes than in striatal regions, as previously reported.[6,30,31] The test-retest variability of [^{11}C]-(+)-PHNO BP_{ND} in SN was larger than in the striatum, ranging from 8.1% to 19% using different [^{11}C]-(+)-PHNO paradigms,[65,66] which is compatible with the larger variation we observed in SN. The negative displacement we found in HV may potentially be explained by test-retest variations. In contrast, the positive displacement in SCZ, is larger than the test-retest variation reported in the literature[65,66] and can be conceivably interpreted as reflecting elevated DA release. Although

automated delineation helps to refine this relatively small ROI more precisely, the spatial resolution of the PET may be compromised in participants with significant movement during PET scans. Additionally, the resolution of the scanner does not permit differentiation of histological subdivisions of SN (ie, SNr and SNc) and nearby structures such as VTA. Another limitation is that [^{11}C]-(+)-PHNO signal in the SN reflects tracer binding to D_3 receptors (100%)[34]; and may not generalize to D_2 receptors studies in the same brain region. Although the current sample size provided sufficient power to detect group differences (partial $\eta^2 = 0.203$, achieved power = 0.93), the relatively small sample size of the SCZ cohort ($n = 9$) limits interpretation of association findings in this subgroup. Finally, the history of substance use may have made the interpretation of the results difficult. However, we controlled by cannabis use, suggesting that it is not a major confounding factor regarding clinical group differences, and further reported significant cannabis effects in [^{11}C]-(+)-PHNO % displacement in non-cannabis users (10.90%) relative to users (−13.22%).

In conclusion, our results of greater stress-induced [^{11}C]-(+)-PHNO % displacement in SN in SCZ suggests that the elevated striatal DA responsivity to stress in SCZ extends to D_3-rich regions in midbrain. The association between SN and striatal [^{11}C]-(+)-PHNO binding changes in non-cannabis-using CHR may reflect an altered relationship between DA activity in SN with DA activity in striatum in this population, but needs further investigation to confirm. Chronic cannabis use is associated with reduced nigral DA response to stress in CHR and SCZ and may mask associations between nigrostriatal DA neurotransmission and clinical presentation.

Supplementary Material

Supplementary material is available at *Schizophrenia Bulletin* online.

Funding

This work was supported by operating grants from the Canadian Institutes for Health Research (CIHR) and the Ontario Mental Health Foundation (OMHF). RM was supported by a New Investigator Award from CIHR and an OMHF New Investigator Fellowship.

Acknowledgments

R.M. has received speaker's honoraria from Otsuka-Lundbeck Canada. No other disclosure is declared by all authors. We are grateful to Armando Garcia, Winston Stableford, Alvina Ng, Terry Bell, Ted Harris-Brandts and Peter Bloomfield.

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