Research Paper

Dopamine D_2 and D_3 binding in people at clinical high risk for schizophrenia, antipsychotic-naive patients and healthy controls while performing a cognitive task

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Background: The dopamine (DA) D_2 receptors exist in 2 states: a high-affinity state (D_2^{high}) that is linked to second messenger systems, responsible for functional effects, exhibits high affinity for agonists (e.g., DA), and a low-affinity state that is functionally inert exhibits lower affinity for agonists. The DA D_3 receptor subtype exhibits high agonist affinity, whereas the existence of the multiple affinity states is controversial. Preclinical studies in animal models of psychosis have shown a selective increase of D_2^{high} as the common factor in psychosis, and the D_3 receptor has been suggested to be involved in the pathophysiology of schizophrenia. **Methods:** We studied D_2^{high} and D_3 in people at clinical high risk (CHR) for schizophrenia and in antipsychotic-naive patients with schizophrenia using the novel positron emission tomography radiotracer, [^{11}C]-(+)-PHNO. The binding potential nondisplaceable (BP_{ND}) was examined in the regions of interest (ROI; caudate, putamen, ventral striatum, globus pallidus, substantia nigra and thalamus) using an ROI and a voxel-wise approach while participants performed a cognitive task. **Results:** We recruited 12 CHR individuals and 13 antipsychotic-naive patients with schizophrenia-spectrum disorder, whom we compared with 12 age- and sex-matched healthy controls. The BP_{ND} between patients and controls did not differ in any of the ROIs, consistent with the voxel-wise analysis. Correlations between the BP_{ND} in D_3-rich regions and psychopathology warrant further investigation. **Limitations:** In the absence of resting-state (baseline) BP_{ND} data, or following a depletion paradigm (i.e., α-methyl partyrosine), it is not possible to ascertain whether the lack of difference among the groups is owing to different levels of baseline DA or to release during the cognitive task. **Conclusion:** To our knowledge, the present study represents the first effort to measure the D_2 and D_3 receptors under a cognitive challenge in individuals putative/prodromal for schizophrenia using [^{11}C]-(+)-PHNO.

Introduction

It is well established that the $D_{2/3}$ dopamine (DA) receptor subtypes are the targets of most antipsychotic drugs, though the exact role of these receptors in the pathophysiology of schizophrenia remains unclear.

Most studies investigating DA D_2 receptor binding could not find any difference between healthy controls and patients with schizophrenia,[1–3] although meta-analyses have shown moderate effects,[4,5] consistent with the early report by Wong and colleagues.[6] However, the D_2 receptor exists in 2 interconvertible states: a G protein–coupled state, which has a high affinity for agonist binding and is responsible for the functional effects of DA, and a functionally inert state with a low affinity for DA. Data from more than a dozen preclinical animal models of psychosis (e.g., amphetamine sensitization, phencyclidine sensitization, ethanol withdrawal, hippocampal lesion)[7] suggest that there is a selective elevation of high-affinity states in psychosis. Molecular imaging techniques, such as positron emission tomography (PET), allow for the in vivo study of brain receptors; however, until recently the available tracers for imaging $D_{2/3}$ receptors (e.g., [^{11}C]-raclopride, [^{18}F]-fallypride) were all "antagonist" radioligands that could not distinguish between the high- and low-affinity states of the

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receptors. Studying the high-affinity state of the receptor directly in patients requires the use of an "agonist" radioligand.

[^{11}C]-(+)-4-propyl-9-hydroxynaphthoxazine ([^{11}C]-(+)-PHNO) is a $D_{2/3}$ agonist radiotracer[8] that binds to D_2 and D_3 receptors, resulting in prominent tracer uptake in the striatum, globus pallidus,[9] thalamus and substantia nigra.[10,11] It exhibits more than 10-fold higher affinity for D_3 than D_2 in vitro, and an estimated 20-fold higher affinity in vivo.[12] This preferential binding to D_3 has been confirmed ex vivo in rodents[13] and in vivo in nonhuman primates and humans.[10–12] Whereas [^{11}C]-(+)-PHNO binds preferentially to D_3, the overall regional tracer retention is a function of the differential D_2 and D_3 subtype affinity and the concentration of D_2 versus D_3 receptors in a given region. In D_3-rich regions like the globus pallidus and substantia nigra, where D_3 binding is thought to account for about 65% and 100% of the [^{11}C]-(+)-PHNO specific signal, respectively, its signal can be largely a marker of D_3 effects. In other regions, such as the dorsal striatum (caudate and putamen), the relative concentration of D_2 receptors is much higher, and therefore only a small component of the signal under 10% is attributable to D_3.[10,11] The ventral striatum and the thalamus represent $D_{2/3}$ mixed regions, as 26% and 43%, respectively, of their signal represents D_3 binding. Consequently, the globus pallidus and substantia nigra can be used as a D_3 preferential binding region and the caudate–putamen as a D_2 preferential region. Thus, the use of [^{11}C]-(+)-PHNO provides an opportunity to study D_2 in the high-affinity state (although see the study by Seeman[14]) and D_3 receptors by comparing caudate–putamen versus globus pallidus–substantia nigra in the same individual.

[^{3}H]-PHNO binding is nearly fully inhibited by guanine nucleotide, a substance known to convert D_2^{high} into D_2 low, providing direct evidence that [^{3}H]-PHNO selectively binds to D_2 receptors in the high-affinity states in vitro.[15,16] Although the existence of D_2 receptors in 2 affinity states in vivo remains controversial (see studies by Seeman[14] and by McCormick and colleagues[17]), as an agonist, [^{11}C]-(+)-PHNO is more sensitive to the DA releasing effect of amphetamine than to antagonist radioligands in humans.[18] Thus, in addition to having the advantage of examining both D_2 and D_3 receptors in the same individual, the use of [^{11}C]-(+)-PHNO provides us with a superior tool to measure changes in endogenous DA levels.

A recent study using [^{11}C]-(+)-PHNO could not find any difference at the DA D_2 and D_3 receptors between healthy controls and patients with schizophrenia.[19] However, the study was carried out while participants were in a resting state, whereas DA function is highly dependent on the environment. Recent data comparing resting, cognitive task and motor task binding potentials showed striatal DA release occurs during behavioural challenges, but not in a resting condition.[20] This is consistent with the well-replicated finding of increased amphetamine-induced DA release in schizophrenia,[21,22] whereas no differences were reported between patients with schizophrenia and healthy controls in the resting state.[1–3,6] Thus, examining $D_{2/3}$ receptor binding under a cognitive challenge, rather than under a resting state, will provide a first hint of altered striatal DA function between healthy controls, medication-naive patients with schizophrenia and individuals at clinical high risk (CHR) for schizophrenia (putative/prodromal for schizophrenia).

Furthermore, to our knowledge, the state of the DA D_2 and D_3 receptors during a cognitive task in CHR individuals has never been tested with either an antagonist or an agonist radioligand. Importantly, established criteria for the identification of people prodromal for schizophrenia have only very recently been attained.[23] Unlike studies involving first-degree relatives of patients with schizophrenia or those with schizotypal personality disorder, CHR studies have enabled prospective identification of individuals at high risk for an imminent onset of psychosis.[24] In the past decade, the CHR (or "prodromal," "ultra high risk" or "at risk mental state") group has become a reliably identifiable clinical constellation with clear and compelling power to predict the onset of psychosis within the near future (1–2 yr).[24]

The aim of the present study was to compare DA D_2 and D_3 receptor binding among healthy controls, CHR individuals and antipsychotic-naive patients with schizophrenia while performing a cognitive task.

Methods

Participants

Individuals at CHR for schizophrenia, medication-naive patients with schizophrenia-related disorders and healthy controls matched for age and sex were recruited for participation in this study. We excluded individuals with a current diagnosis of substance abuse or dependence at the time of screening, history of clinically important physical illness or metal implants that would preclude the magnetic resonance imaging (MRI) scan. We also excluded women who were pregnant or lactating at the time of screening or who had a positive urine pregnancy test before the PET scan. Among our participants, 12 controls, 12 CHR individuals and 10 medication-naive patients with schizophrenia-related disorders were included in the sample of a previous study.[25] Participants provided written informed consent after the study procedures and risks were explained. Our study protocol was approved by our local research ethics board.

Criteria for CHR include the following: attenuated positive symptoms syndrome, the genetic risk and deterioration syndrome and the brief intermittent psychosis syndrome. Participants meeting criteria for prodromal syndromes based on the Structured Interview for Prodromal Symptoms (SIPS) and the Scale for the Assessment of Prodromal Symptoms (SOPS)[26] were recruited from the prodromal clinic at the Centre for Addiction and Mental Health, Toronto, Ont. Participants were either self-referred or referred by health care providers, educators or social service agencies in response to intensive community education efforts, including academic detailing, grand rounds, educational talks, mailings, advertisements, website hits and public service announcements. Matched healthy controls from the same geographic area, socioeconomic status and race as the patients were recruited through advertisements.

We corroborated the diagnosis of schizophrenia or schizophreniform disorder in the patient group using the Structured Clinical Interview for DSM-IV (SCID-I),[27] which was carried out by a psychiatrist (R.M.). Participants were asked to consume no more than their usual amount of coffee and nicotine, if applicable, on the day of the PET scan and to abstain from alcohol intake for 24 hours before PET scans.

Patients were evaluated before the PET scan using the Clinical Global Impression[28] and Positive and Negative Syndrome Scale (PANSS)[29] in the patient group and the SOPS in the CHR group.

Cognitive task

Participants completed the cognitive task while lying inside the PET scanner. The task consisted of 6 blocks of mental arithmetic tests lasting 6 minutes each, involving addition, subtraction, multiplication and division. Participants were told to provide as many correct answers as possible in the 6-minute timeframe but to take as much time as needed to answer each question correctly. Participants viewed the screen with a pair of goggles connected to a computer. The computer screen displayed the arithmetic questions, a numeric response button and performance ratings ("correct," "incorrect," "not a number") for each question. Participants answered by clicking on the correct number using a mouse. A performance bar divided into red, yellow and green zones was displayed at the top of the screen. Participants were told to perform so that the arrow indicating their performance rating on the task would stay in the green zone. To reduce the level of stress possibly induced by the task, all participants were asked to perform 2 trials lasting 6 minutes each before the scan day to ensure understanding and reduce the effects of novelty. All participants were allowed to take as much time as needed to answer the questions correctly and were encouraged to concentrate on the task, but not on the answers. The outcomes noted for each participant were the the number of mathematical tasks completed and number of errors committed. The task has been previously described by Pruessner and colleagues,[30] although in the present study we report only on the sensory motor control task, not the stress task component.

[^{11}C]-(+)-PHNO synthesis

The radiosynthesis of [^{11}C]-(+)-PHNO has been described in detail elsewhere.[8] Briefly, [^{11}C]-propionyl chloride reacted with 9-hydroxynaphthoxazine to generate a [^{11}C]-amide, which is subsequently reduced by lithium aluminum hydride. Purification by high performance liquid chromatography and formulation in saline gave radiochemically pure [^{11}C]-(+)-PHNO as a sterile, pyrogen-free solution suitable for human studies.

PET imaging

Studies were carried out using a high-resolution PET-CT imaging system (Siemens-Biograph HiRez XVI; Siemens Molecular Imaging), which measures radioactivity in 81 brain sections with a thickness of 2.0 mm each. The images were reconstructed with a 2-dimentional filtered back projection algorithm with a ramp filter at Nyquist cut-off frequency. A custom fitted thermoplastic mask was made for each participant and used with a head fixation system during PET measurements. Scanning time was 90 minutes.

MRI scanning

We obtained a proton density image (echo time [TE] 17 ms, repetition time [TR] 6000 ms, field of view [FOV] 22 cm, matrix 256 × 256, slice thickness 2 mm, number of acquisitions 2) for each participant using a 1.5 T Signa scanner (General Electric Medical Systems). These images were used for the analysis of the PET scans.

Image analysis

Using the participants' MRI scans, we divided the striatum into the caudate, putamen and ventral striatum based on a set of landmarks, as described previously.[31,32] In addition, we delineated the substantia nigra and thalamus, as described previously.[11] Time activity curves from the regions of interest (ROIs) were obtained from the dynamic [^{11}C]-(+)-PHNO PET images. Activity from the right and left regions were averaged together, and we used a weighted average (weighted by subregion volume) to derive nondisplaceable binding potentials (BP_{ND}). We delineated these ROIs using an automated method implemented in an in-house software region of mental interest (ROMI), abolishing subjectivity in manual ROI drawing.[33] Briefly, ROMI includes the following steps: (1) a standard brain template with a set of predefined ROIs is nonlinearly transformed to match the individual high-resolution MRI scan; (2) the ROIs from the transformed template are refined based on the grey matter probability of voxels in the individual MRI; and (3) the individual MRI is registered to the PET images so that the individual refined ROIs are transformed to the PET image space, allowing the time activity curves generation from each ROI. To obtain a quantitative estimate of binding, we analyzed the time activity curves using the simplified reference tissue model (SRTM).[34] The SRTM uses a within-brain reference region (cerebellum in this case) instead of arterial input. It provides an estimate of the BP_{ND} of the radiotracer, which is proportional to the more fundamental parameters of receptor number (Bmax) and affinity (1/Kd) [$BP \approx Bmax/Kd$]. This method has previously been validated for BP_{ND} with [^{11}C]-(+)-PHNO.[35] The BP_{ND}s were estimated using the PMOD software version 2.7 (PMOD Technologies Ltd.).

Voxel-wise images of [^{11}C]-(+)-PHNO binding were generated using a data-driven method with reference region implemented in the data-driven estimation of parametric images based on compartmental theory,[36] which requires no compartmental model assumptions or a priori anatomic hypotheses (i.e., ROI definition). We used a subregion of the cerebellar cortex devoid of $D_{2/3}$ receptors to generate the time activity curve of the reference region. Specifically, the cerebellar vermis was excluded, and only the grey matter of the cerebellum was included as the reference region. Each

parametric map was spatially normalized to the Montreal Neurological Institute (MNI) anatomic template using SPM2 normalization and coregistration tools. Once normalized, BP_{ND} maps were used to assess clinically important contrasts between groups (medication-naive, CHR, control) at the level of the whole brain using an implicit mask of $BP_{ND} > 0.3$. This mask restricts the statistical search to areas of specific binding (i.e., excluding cerebrospinal fluid, background and the reference region). Differences among the groups were tested using F test analysis, as implemented in SPM5, followed by an independent *t* test to investigate group contrasts.

Statistical analysis

We performed statistical analyses using SPSS version 17.0. Variables were presented as means and standard deviations. Once we obtained the [^{11}C]-(+)-PHNO BP_{ND} for each striatal region, we tested group differences using repeated-measures analysis of variance (RM-ANOVA) with ROI as a within-subjects factor and study group as a between-subjects factor. The Bonferroni correction for multiple comparisons was used post hoc to investigate differences among the CHR, medication-naive and control groups. Pearson's product moment correlations between PANSS (total and subscales), SOPS, performance on the cognitive task and BP_{ND} were explored. We considered results to be significant at $p < 0.05$.

Results

We included 37 participants in our study: 12 healthy controls, 12 CHR individuals and 13 medication-naive patients with schizophrenia-related disorders. The demographic and clinical characteristics of participants are summarized in Table 1. There were no differences between groups for any variable except, as expected, for mean task completion ($F_{2,36} = 7.23$, $p = 0.002$), where healthy controls completed significantly more mathematical questions than CHR individuals and medication-naive patients (Bonferroni-corrected $p = 0.007$ and $p = 0.006$, respectively), with no significant difference between CHR individuals and medication-naive patients. Smoking status (Fisher exact test = 5.76, $p = 0.06$) and cannabis use (Fisher exact test = 4.19, $p = 0.09$) showed a trend level of significance between medication-naive patients and both CHR individuals and healthy controls. No differences emerged between BP_{ND} of CHR individuals, medication-naive patients and healthy controls in any of the ROIs (group $F_{2,33} = 0.47$, $p = 0.62$), with a significant region effect (region $F_{5,29} = 206.81$, $p < 0.001$) but no significant interaction (region × group $F_{10,58} = 1.34$, $p = 0.23$). Results remained when participants who smoked tobacco (group $F_{2,25} = 1.38$, $p = 0.27$; region $F_{5,21} = 125.64$, $p < 0.001$; group × region $F_{10,42} = 2.43$, $p = 0.017$) or cannabis (group $F_{2,29} = 0.76$, $p = 0.48$; region $F_{5,25} = 216.69$, $p < 0.001$; group × region $F_{10,50} = 1.97$, $p = 0.06$) were excluded from the analysis. Univariate tests showed a weak trend toward a significant difference among the groups at the level of the caudate ($F_{2,34} = 2.88$, $p = 0.07$; Fig. 1), and a significant difference in the globus pallidus when participants who smoked tobacco were excluded from the analysis ($F_{2,26} = 4.07$, $p = 0.029$). The cerebellar uptake in CHR individuals, medication-naive patients with schizophrenia and sex- and age-matched healthy controls was superimposable (Fig. 2). The overall lack of BP_{ND} difference among the groups was

Table 1: Characteristics of healthy controls, people at clinical high risk for schizophrenia and medication-naive patients with schizophrenia*

	Group; mean (SD)†		
Characteristic	Control, *n* = 12	CHR, *n* = 12	Schizophrenia, *n* = 13
Age, yr	26.1 (3.8)	23.00 (4.6)	23.38 (4.6)
Sex, no.			
Female	5	5	3
Male	7	7	10
[11C]-(+)-PHNO			
Amount injected	8.97 (1.66)	9.17 (2.15)	9.41 (1.30)
Specific activity	1019.76 (453.50)	1014.84 (434.26)	1076.51 (477.06)
Mass injected	2.28 (0.75)	2.58 (0.90)	2.43 (0.75)
SOPS-P score	NA	11.25 (2.90)	NA
PANSS-P score	NA	NA	18.92 (4.48)
Mean task completion	44.23 (12.15)	24.52 (15.08)	24.59 (16.43)
Mean task errors	5.23 (3.38)	5.16 (3.38)	8.86 (8.03)
Smoking status, no.			
Nonsmoker	11	11	7
Smoker	1	1	6
Cannabis use, no.			
No	0	0	9
Yes	0	0	4

CHR = clinical high risk for schizophrenia; NA = not applicable; P = positive; PANSS = Positive and Negative Symptom Scale;[29] SD = standard deviation; SOPS = Scale for the Assessment of Prodromal Symptoms.[26]
*There was no difference among the groups in any variable, except mean task completion ($F_{2,36} = 7.23$, $p = 0.002$), and there were trend-level differences for smoking status (Fisher exact test = 5.76; $p = 0.06$) and cannabis use (Fisher exact test = 4.19, $p = 0.09$).
†Unless otherwise indicated.

corroborated by the voxel-wise comparison of the BP_{ND} maps between CHR individuals and medication-naive patients and controls, which did not show any significant differences (Fig. 3). Exploration of the clinical/cognitive data correlations with BP_{ND} for each region is presented in Table 2.

Discussion

To our knowledge, this study represents the first comprehensive investigation of the DA D_2^{high} and D_3 in individuals at CHR for schizophrenia. In agreement with a previous study involving patients with first-episode schizophrenia in a resting-state condition, we found no significant evidence of increased D_2^{high} or D_3 binding among our study groups.[19] Moreover, to our knowledge, we present the first observations that CHR individuals showed no elevation in D_2^{high} or D_3, consistent with the patient population, even while performing a cognitive task. The present results are also corroborated by voxel-wise analysis. We found, however, interesting associations between clinical variables and D_3 binding that would need to be further explored in future studies.

There has been an ongoing debate regarding whether there is a difference in $D_{2/3}$ receptor densities between patients with schizophrenia and healthy controls.[37] In one study using [^{11}C]-methylspiperone, a significant elevation in $D_{2/3}$ receptor densities was observed in patients with longer duration of illness;[6] whereas in another study involving patients with shorter duration of illness, no significant difference was found, as determined by [^{11}C]-Raclopride.[38] Some of the patients with schizophrenia included in the latter study did not meet the DSM-III severity criteria for the disease during the study period, and most had a relatively short duration of illness. Given the similarity to their patient population, our findings are consistent with those of the latter study. However, it is important to note that comparisons among these studies are limited owing to the use of different PET radioligands and the quantification of $D_{2/3}$ receptor density during a cognitive challenge rather than under a resting state. A recent meta-analysis, however, has shown that increased $D_{2/3}$ receptor densities are found when all baseline studies are pooled.[4,5]

An initial report from Koepp and colleagues[39] showed that a behavioural task, which had an important motor component, was associated with DA release in healthy individuals. However, the authors later reported that the effect was not significant after images were corrected for head movement, which may suggest that DA function was not related to the behavioural task. However, as recently shown by Lappin and colleagues,[20] it is important to separate the influence of different behavioural task components in the analysis of DA function. In the present study, we used a task that tapped into working memory and attention (e.g., participant must remember particular numbers while computing the right answers), whereas the behavioural task used by Koeep and colleagues[39] tapped less into these cognitive processes (i.e., their behavioural task involved earning points for moving a tank through a battlefield on a screen using a mouse).

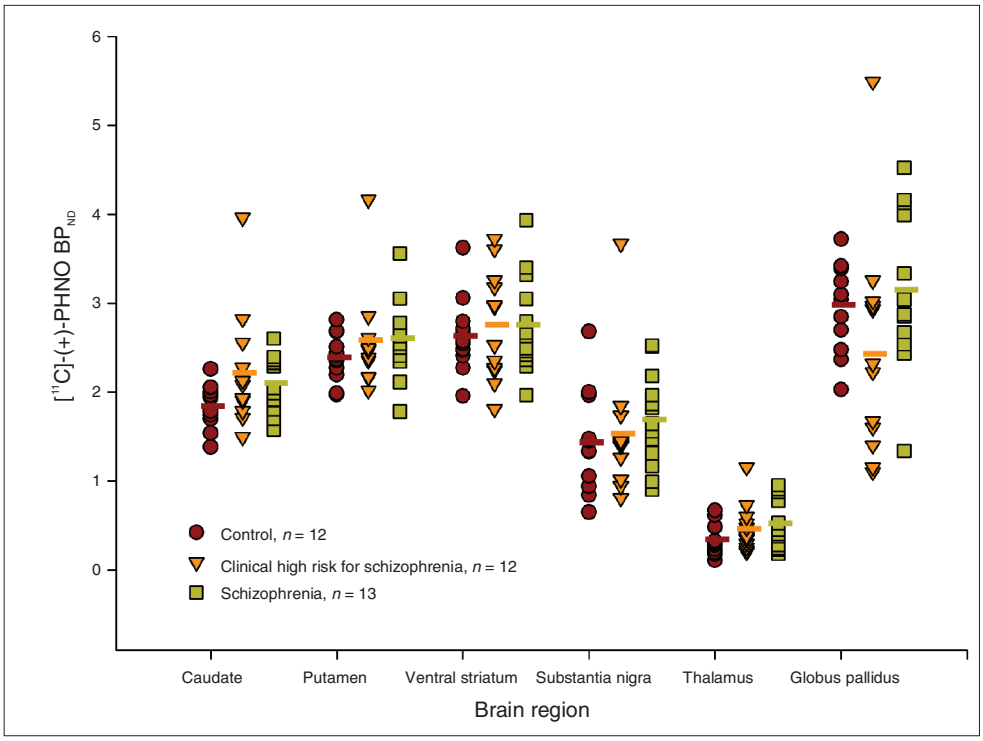

Fig. 1: [^{11}C]-(+)-PHNO positron emission tomography scans of binding potential nondisplaceable (BP_{ND}) in the caudate, putamen, ventral striatum, substantia nigra, thalamus and globus pallidus, showing no significant difference among the groups in any brain region studied. Horizontal bars represent means for each group.

The high-affinity state hypothesis has been sustained in many preclinical models of psychosis[7] and has been indirectly suggested by DA depletion studies in patients with schizophrenia.[40] However, our results in CHR individuals and in medication-naive patients are at odds with this preclinical data. First, there is ongoing debate regarding the measurability of DA receptor high-affinity state in vivo. For example, Sibley and colleagues[41] identified both high- and low-affinity binding sites in membrane cells, but only low-affinity sites on the same intact cells. However, Seeman[42] identified the high-affinity binding sites on intact cells with [^{3}H]domperidone, but not with [^{3}H]spiperone or [^{3}H]raclopride. More recent reports even suggest [^{11}C]-(+)-PHNO may not be suitable to measure D_2^{high} in vivo.[14] Moreover, one PET study in nonhuman primates with [^{11}C]-NPA (D_2 agonist, (-)-N-[^{11}C]Propyl-norapomorphine) and [^{11}C]-raclopride ($D_{2/3}$ antagonist) through a Scatchard plot analysis was able to differentiate the density of receptors (Bmax) in the high- versus high+low-affinity state,[43] although the same approach in cats with [^{11}C]-(+)-PHNO and [^{11}C]-raclopride did not find any difference in the Bmax between the 2 radiotracers.[44] On the other hand, studies in anesthetized cats,[44] anesthetized nonhuman primates[45,46] and awake humans[18,47,48] showed greater displacement with an agonist radiotracer ([^{11}C]-NPA, [^{11}C]-MNPA (D_2 agonist), 3-(methylnitrosamino)propionaldehyde, [^{11}C]-(+)-PHNO) than with an antagonist radiotracer in response to an amphetamine challenge (alternatively, see negative findings with apomorphine in the study by Finnema and colleagues[49]). Consistent with this last study, an ex vivo study in awake rodents did not find any difference in amphetamine displacement between [^{11}C]-(+)-PHNO and [^{11}C]-raclopride.[17] These studies suggest that, although agonist radiotracers do show a distinct binding profile in vivo compared with in vitro, the exact reasons for these divergent results are still unclear. Importantly, the current data are also consistent with that from previous studies using antagonist radioligands, which showed no difference in D_2 binding between healthy controls and patients with schizophrenia,[4,5,50] although in those studies, participants were scanned at rest. In contrast, several studies reported reduced $D_{2/3}$ in the thalamus in patients with schizophrenia,[51–53] whereas Kegeles and colleagues[54] observed increases in thalamic BP_{ND}. Consistent with the results of Glenthoj and colleagues[55] and Talvik and colleagues,[56] we found no significant difference at the level of the whole thalamus.

Limitations

It is theoretically possible that an increase in D_2^{high} is present in CHR individuals and in patients with schizophrenia, but this may be masked by the presence of elevated levels of DA in response to the cognitive task,[39] although it is currently unclear if these effects would be different in CHR individuals, patients with schizophrenia and healthy controls when using an agonist radioligand. The abnormally high level of DA in psychosis has been indirectly shown after an amphetamine challenge in patients with schizophrenia[21,22] and in CHR individuals after a stress challenge.[25] Consistent with these data, DA striatal synthesis capacity has been found to be higher in patients with schizophrenia than in controls in most studies.[57–60] More recently, Howes and colleagues[61] also

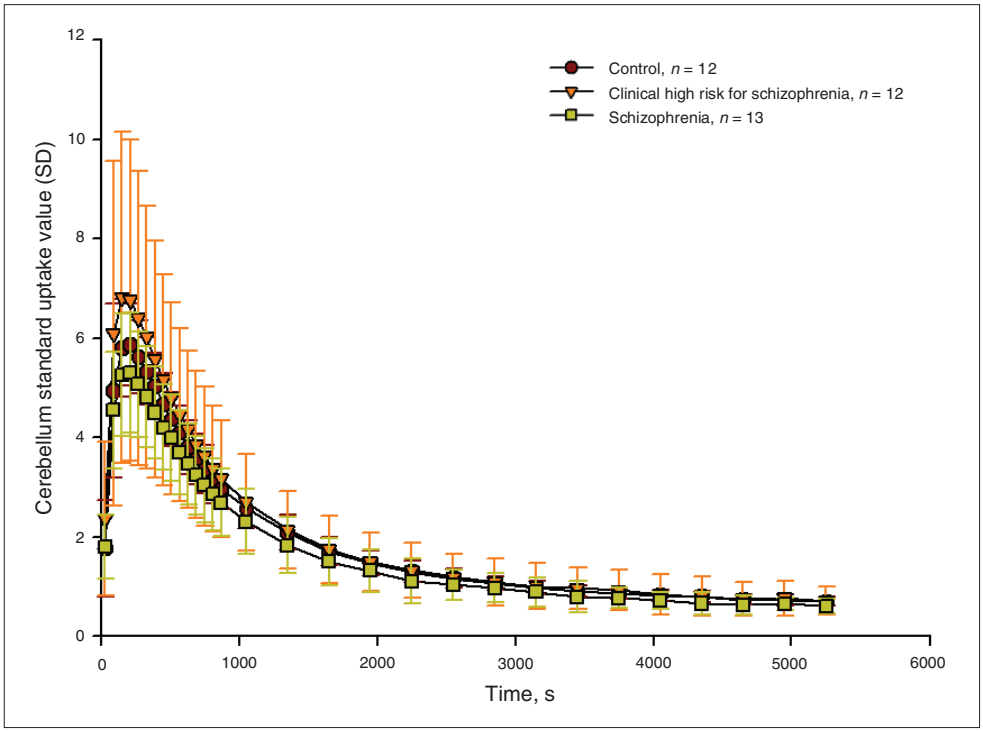

Fig. 2: [^{11}C]-(+)-PHNO cerebellar time-activity curves (expressed as standard uptake value and standard deviation [SD]) are superimposable among the groups.

reported increased DA synthesis capacity in both CHR individuals and patients with schizophrenia, which correlated with conversion to schizophrenia.[62] In the absence of resting-state (baseline) BP_{ND} data, or following a depletion paradigm (i.e., α-methyl partyrosine), it is not possible to ascertain whether the lack of difference among the groups is owing to different levels of baseline DA or to release during the cognitive task. In an attempt to solve this problem, we compared the present BP_{ND} values with baseline (resting) BP_{ND} data from a previous study in patients with schizophrenia.[63] We found no significant difference between the baseline BP_{ND} reported in our previous study[19] and the ones observed under cognitive activation in the present study in all of the ROIs tested (group $F_{1,19} = 0.735$, $p = 0.40$; region $F_{4,16} = 21.821$, $p < 0.001$; group × region $F_{4,16} = 1.014$, $p = 0.43$). In addition, the lack of difference in [^{11}C]-(+)-PHNO binding among the groups may reflect the small DA releasing effect of this particular cognitive task or the lack of participants' own resting state baselines, which may have translated into a statistically nonsignificant difference among the groups.

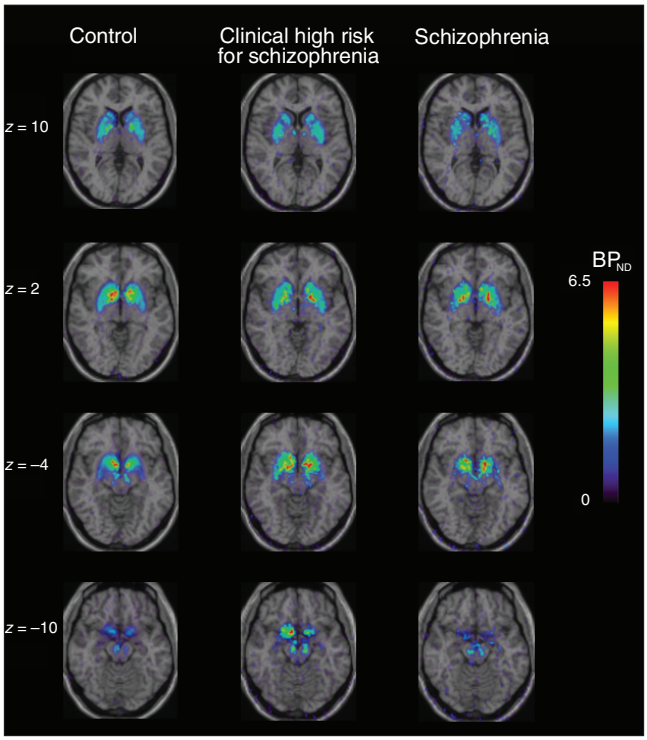

Fig. 3: Voxel-wise comparison of the [^{11}C]-(+)-PHNO binding potential nondisplaceable (BP_{ND}) maps between people at clinical high risk for schizophrenia, medication-naive patients with schizophrenia and healthy controls, showing no significant differences.

Another issue to consider is that the [^{11}C]-(+)-PHNO signal reflects tracer binding to both D_2 and D_3 receptor populations. The contribution of each receptor to the detected regional distribution varies according to the relative presence of each receptor subtype in the region and to the affinity of the radioligand for that receptor subtype. Although the precise contribution of D_2 and D_3 to each ROI is not fully mapped, studies in rodents, baboons and humans estimate that the vast majority of the [^{11}C]-(+)-PHNO binding in the globus pallidus (~68%) and in the midbrain substantia nigra (100%) corresponds to D_3.[11] On the other hand, the estimation of [^{11}C]-(+)-PHNO binding in the dorsal striatum that corresponds to D_3 is below 10%.[11] There is the hypothetical possibility that a simultaneous increase in D_2, coupled with a decrease in D_3 (or vice versa) could conceal any change in tracer retention. There are, however, no data in the literature to suggest such a possibility, and data from the substantia nigra, which is 100% D_3,[11] would contradict this possibility.

The lack of a full kinetic analysis is another potential limitation. This approach would have allowed for the direct estimation of the BP_{ND} in the ROIs, without the assumption of equivalent nonspecific and free fraction in the reference region. Whereas our data showed that the cerebellar uptake in CHR individuals, medication-naive patients with schizophrenia and sex- and age-matched healthy controls was superimposable (Fig. 2) and, therefore, unlikely that a true finding could have been obscured by differences in the reference region, without arterial data this cannot be answered with certainty. In addition, as described in the study by Ginovart and colleagues,[35] the binding potential of [^{11}C]-(+)-PHNO can be reliably quantified using the SRTM, with cerebellum as a reference region. Finally, the sample size of the present study

Table 2: Associations between [11C]-(+)-PHNO binding potential nondiplaceable in different regions of interest and clinical and cognitive data, $p < 0.05$

	Region of interest; r (*p* value) [no.]					
Scale	Caudate	Putamen	Ventral striatum	Globus pallidus	Thalamus	Substantia nigra
Total PANSS P	–0.029 (0.92) [13]	–0.224 (0.46) [13]	–0.203 (0.51) [13]	–0.190 (0.52) [13]	0.459 (0.13) [13]	0.626 (0.022) [13]*
Total PANSS N	0.311 (0.30) [13]	0.518 (0.07) [13]	0.195 (0.52) [13]	–0.035 (0.91) [13]	0.006 (0.99) [13]	–0.021 (0.95) [13]
Total PANSS G	–0.076 (0.80) [13]	0.261 (0.39) [13]	0.286 (0.34) [13]	–0.091 (0.77) [13]	–0.004 (0.99) [13]	0.057 (0.85) [13]
Total SOPS P	0.261 (0.41) [12]	0.409 (0.19) [12]	0.204 (0.52) [12]	0.389 (0.21) [12]	0.244 (0.44) [12]	0.431 (0.16) [12]
Total SOPS N	–0.006 (0.98) [12]	–0.009 (0.98) [12]	–0.228 (0.48) [12]	–0.125 (0.70) [12]	0.105 (0.74) [12]	–0.357 (0.25) [12]
Total SOPS D	0.452 (0.14) [12]	0.384 (0.22) [12]	0.2 (0.53) [12]	0.234 (0.46) [12]	0.256 (0.42) [12]	0.182 (0.57) [12]
Total SOPS G	0.52 (0.08) [12]	0.580 (0.048) [12]*	0.513 (0.09) [12]	0.451 (0.14) [12]	0.413 (0.18) [12]	0.359 (0.25) [12]
Mean task completion	–0.271 (0.10) [37]	–0.118 (0.49) [37]	0.078 (0.65) [37]	–0.080 (0.64) [37]	0.019 (0.91) [37]	–0.223 (0.18) [37]
Mean no. errors	0.015 (0.93) [37]	0.109 (0.52) [37]	–0.141 (0.41) [37]	0.195 (0.25) [37]	0.200 (0.24) [37]	0.253 (0.13) [37]

D = disorganization; G = global; N = negative; NA = not applicable; P = positive; PANSS = Positive and Negative Symptom Scale;[29] SD = standard deviation; SOPS = Scale for the Assessment of Prodromal Symptoms.[28]
*Correlations did not survive Bonferroni correction for multiple comparisons.

was relatively small. Although we observed a weak trend toward a significant difference in $D_{2/3}$ binding in the caudate and globus pallidus, a larger sample size may have allowed us to detect a significant difference in other regions. Nevertheless, this study provides preliminary data on the status of $D_{2/3}$ binding under a cognitive task in CHR individuals and patients with schizophrenia. In addition, the correlation between substantia nigra BP_{ND} and positive symptoms of the illness would not surpass Bonferroni correction. However, given that this is the first report of the potential contribution of D_3 to psychopathology, it should be regarded as exploratory and possibly useful for sample size estimations for future studies.

Conclusion

The present study represents, to our knowledge, the first effort to measure the D_2 and D_3 receptors during a cognitive challenge in medication-naive individuals at CHR for schizophrenia and in medication-naive patients with schizophrenia. Our study does not support that CHR individuals and patients with schizophrenia exhibit an increase in the D_2^{high} or D_3 receptors in comparison to sex- and age-matched healthy controls while performing a cognitive task. Our findings add to the ongoing debate regarding the possible involvement of DA in cognition among individuals with schizophrenia-related disorders. However, the lack of differences among our study groups may be owing to the lack of participants' own resting state baseline values. Future studies should include a 2-scan paradigm to confirm this finding. In addition, an extension to the investigation of these phenomena in cortical regions would further help us to understand the role of DA in the cognitive function of patients with schizophrenia and CHR individuals. The lack of difference in $D_{2/3}$ receptor binding among healthy controls, CHR individuals and patients with schizophrenia under a cognitive task would need to be replicated in the future studies with a larger sample size before definite conclusions can be drawn regarding the status of the $D_{2/3}$ receptors in patients with schizophrenia and CHR individuals.

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Contributors: J. Addington and R. Mizrahi designed the study. I. Suridjan and J. Addington acquired the data, which all other authors analyzed. I. Suridjan and R. Mizrahi wrote the article, which all authors reviewed and approved for publication.

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