Test–Retest Reproducibility of [^{11}C]-(+)-Propyl-Hexahydro-Naphtho-Oxazin Positron Emission Tomography Using the Bolus plus Constant Infusion Paradigm

Dianne E. Lee, Jean-Dominique Gallezot, Ming-Qiang Zheng, Keunpoong Lim, Yu-Shin Ding, Yiyun Huang, Richard E. Carson, Evan D. Morris, and Kelly P. Cosgrove

Abstract

We examined the reproducibility of using the constant infusion paradigm for equilibrium measurement of $D_{2/3}$ receptors using [^{11}C]-(+)-propyl-hexahydro-naphtho-oxazin (PHNO) positron emission tomography (PET). Six subjects were scanned with a bolus plus constant infusion ($K_{bol} = 80$ minutes) of [^{11}C]-(+)-PHNO. Binding potential (BP_{ND}) was computed using the equilibrium approach and compared to a simplified reference tissue model (SRTM). The rate of change in the concentration-activity curve from 60 to 90 minutes was $-5 \pm 13\%/h$ in the caudate, putamen, substantia nigra, thalamus, and cerebellum but was $15 \pm 15\%/h$ in the ventral striatum and pallidum. Test–retest variability was lower in striatal compared to extrastriatal regions ($4 \pm 8\%$ vs $-8 \pm 22\%$, respectively) using the equilibrium approach, with comparable results with SRTM. The equilibrium ratio and SRTM yielded reliable BP_{ND} estimates (intraclass correlation coefficient = 0.88 and 0.82, respectively). These studies support the reproducibility of the bolus plus constant infusion paradigm with [^{11}C]-(+)-PHNO PET.

[^{11}C]-(+)-propyl-hexahydro-naphtho-oxazin (PHNO) is a high-affinity $D_{2/3}$ agonist radiotracer used for positron emission tomography (PET) brain imaging.[1–4] Unlike other $D_{2/3}$ radiotracers that have higher affinity for D_2 over D_3 receptors (eg, [^{11}C]raclopride, [^{18}F]fallypride, [^{11}C]FLB, [^{11}C]NPA, and [^{11}C]MNPA), the affinity of [^{11}C]-(+)-PHNO is ≈ 40 times higher for D_3 than for D_2 receptors.[5–7] As an agonist, [^{11}C]-(+)-PHNO binds to receptors in the high-affinity state, whereas antagonists bind to both high- and low-affinity receptors.[8,9] The high-affinity $D_{2/3}$ receptors are thought to be more functionally relevant than low-affinity receptors and more susceptible to extracellular changes in endogenous dopamine, which binds primarily to high-affinity $D_{2/3}$ receptors.[1,2] Thus, [^{11}C]-(+)-PHNO may provide a more sensitive measure of functionally relevant $D_{2/3}$ receptors than antagonist radiotracers.

A preliminary study using a bolus administration of [^{11}C]-(+)-PHNO demonstrated that the radiotracer has good test–retest reliability, with variability between scans less than 12.5% across regions.[9] The bolus plus constant infusion method provides several advantages over the bolus-only method of radiotracer administration.[10] Therefore, in the current study, we examined the feasibility and reproducibility of a constant infusion paradigm for examining $D_{2/3}$ receptor availability with [^{11}C]-(+)-PHNO PET.

Materials and Methods

Participants

Six physically and mentally healthy nonsmoker men ($n = 4$) and women ($n = 2$), 31 ± 6 years of age (range 23–36 years) participated in the study. Eligibility was determined as follows: subjects were examined by a study physician to exclude major medical issues or neurologic disorders; electrocardiography, serum chemistries, thyroid function studies, complete blood count, urinalysis, and urine

From the Department of Psychiatry, Yale University School of Medicine; Department of Diagnostic Radiology, Yale PET Center, Yale School of Medicine; and Department of Biomedical Engineering, Yale University, New Haven, CT.

Address reprint requests to: Kelly P. Cosgrove, PhD, Yale University School of Medicine and the VACHS, 950 Campbell Avenue/116A6, West Haven, CT 06516; e-mail: kelly.cosgrove@yale.edu.

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toxicology screening were performed; and the Structured Clinical Interview for the *Diagnostic and Statistical Manual of Mental Disorders* was administered to rule out Axis I psychiatric disorders. Participants had no history of significant medical illness or major head trauma. Females had negative pregnancy tests at intake and on each PET scan day prior to radiotracer injection.

[11C]PHNO PET Imaging

[11C]-(+)-PHNO was prepared as previously described.[11] The specific activity at the end of synthesis was 116 ± 34 MBq/nmol. Each participant had one magnetic resonance imaging (MRI) scan and two [11C]-(+)-PHNO PET scans at least 1 week apart (11 ± 6 days) at the Yale PET Center on the High Resolution Research Tomograph (HRRT) (Siemens/CTI, Knoxville, TN). An initial 6-minute transmission scan using an orbiting ^{137}Cs point source was acquired prior to radiotracer injection for attenuation correction. One venous catheter in the forearm antecubital vein was established for administration of [11C]-(+)-PHNO. The injected dose for the test scan was 562 ± 92 MBq with a mass of 0.06 ± 0.01 μg/kg, and the retest scan dose was 543 ± 170 MBq with a mass of 0.05 ± 0.02 μg/kg. For the radiotracer injection, [11C]-(+)-PHNO was diluted with sterile saline solution and delivered as a bolus plus constant infusion (K_{bol} = 80 minutes) by a syringe pump (Harvard PHD 22/2000, Harvard Apparatus, Holliston, MA). This value of K_{bol} was determined by simulation using measured bolus data as previously described.[12] List mode data were reframed into a dynamic sequence of 33 frames (6 × 30 seconds, 3 × 1 minute, 2 × 2 minutes, 22 × 5 minutes). Dynamic images (207 slices; pixel size = 1.2 mm) were reconstructed using OSEM, including an event-by-event motion correction algorithm (MOLAR; Carson IEEE-NSS[13]). Motion correction was measured using an optical motion tracking tool fastened to the subjects head via a Lycra swimcap (Vicra, NDI Systems, Waterloo, ON).

Image Analysis

Each participant's summed early PET image (0–10 minutes) was used for coregistration to the individual subject's MRI, which was then coregistered to a template MRI (Automated Anatomical Labeling[14] template). Decay-corrected time-activity curves were generated for regions of interest, including the caudate, putamen, ventral striatum, pallidum, substantia nigra, thalamus, and cerebellum (left and right regions of interest were averaged for the caudate, putamen, and substantia nigra).

Outcome Measures

Regional [11C]-(+)-PHNO uptake was quantified as binding potential (BP_{ND}), which is proportional to the density of available receptors. BP_{ND} was calculated by both equilibrium ratios and the simplified reference tissue model (SRTM).[15] The cerebellum was used as a reference region. To assess the quality of equilibrium, the rate of change in activity between 60 and 90 minutes was calculated as the slope divided by the average concentration. Equilibrium ratios were based on an average of the data between 60 and 90 minutes, whereas the SRTM used the full-scan duration of 120 minutes. Simulations were performed where K_{bol} was varied, for example, under- and overestimated, and the error [(measured-true)/true] in estimates of BP_{ND} induced was estimated and the effect on rates of change between 60 and 90 minutes and bias in BP_{ND} in the putamen were determined.

Statistical Analyses

Paired *t*-tests (two-tailed; $p < .05$ was considered statistically significant) were used to compare [11C]-(+)-PHNO injection characteristics, including injected dose, specific activity, and mass between test and retest. Test–retest variability (TRV) was calculated as follows: $2[BP_{test} - BP_{retest}]/[BP_{test} + BP_{retest}] \times 100$. The test–retest consistency was measured with an intraclass correlation coefficient.

Results

Estimation of K_{bol}

The K_{bol} value (the ratio of bolus amount to infusion rate) was determined using the regional time-activity curves from a human [11C]-(+)-PHNO bolus study (120 minutes). The optimal K_{bol} was 80 minutes, which predicted that equilibrium would be achieved by 60 minutes in the caudate, putamen, substantia nigra, and cerebellum (Figure 1).

[11C]-(+)-PHNO Injection Characteristics and Pharmacologic Effects

No statistically significant differences were observed in specific activity or injected mass between test and retest
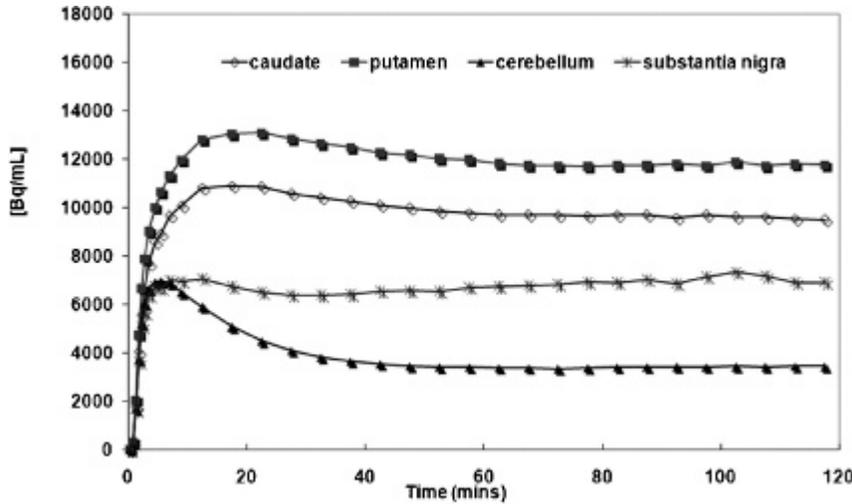
Figure 1. Simulations of predicted tissue response to bolus plus constant infusion of $[^{11}C]$-(+)-PHNO using $K_{bol} = 80$ minutes (curves are decay corrected).

scans. Two of eight enrolled subjects experienced side effects (nausea and emesis), which have been previously reported for $[^{11}C]$-(+)-PHNO.[9,16] In both cases, the infusion and scan were stopped within 5 minutes of the start of injection. The mass dose for the bolus phase of $[^{11}C]$-(+)-PHNO for these subjects (0.02 μg/kg) was within the range of the other subjects (0.01–0.04 μg/kg).

Figure 2. Time-activity curves of bolus plus constant infusion ($K_{bol} =$ 80 minutes) of $[^{11}C]$-(+)-PHNO for test (A) and retest (B) of a representative subject (curves are decay corrected). CB = cerebellum.

Feasibility of the Bolus plus Infusion Paradigm

Time-activity curves (Figure 2) demonstrated low rates of change between 60 and 90 minutes in the caudate ($-4 \pm 13\%/h$), putamen ($-8 \pm 8\%/h$), substantia nigra ($-6 \pm 19\%/h$), thalamus ($-6 \pm 14\%/h$), and cerebellum ($-2 \pm 12\%/h$). However, high rates of change were observed in the ventral striatum ($10 \pm 17\%/h$) and pallidum ($20 \pm 13\%/h$).
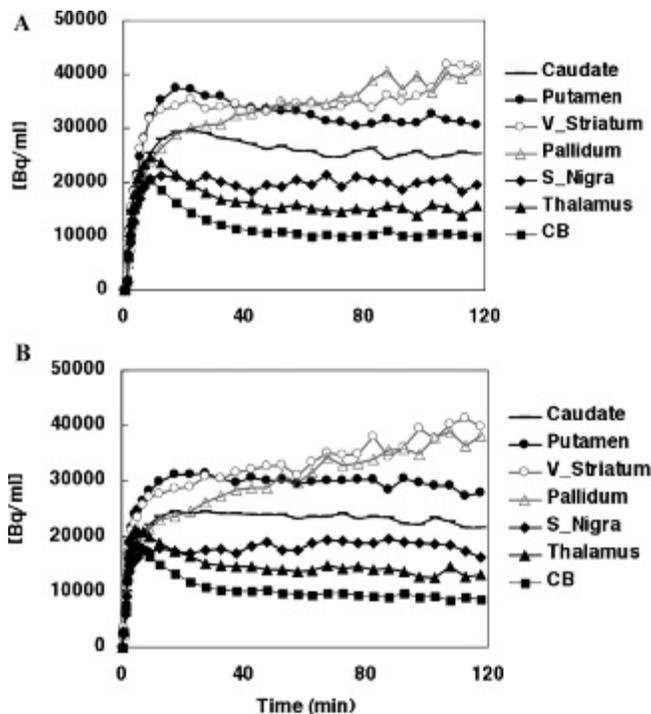
Variability and Reliability of the Test and Retest Scans

The test–retest BP_{ND} estimates using the equilibrium method yielded high interscan correlation coefficients ($r^2 = .98$) in all regions. Similarly, the interscan correlation coefficients of BP_{ND} using SRTM across all regions were high ($r^2 = .97$).

The rank order of BP_{ND} (ventral striatum > pallidum > putamen > caudate > substantia nigra > thalamus) was consistent for the equilibrium and SRTM methods. The mean BP_{ND} estimates were similar on both scan days using the equilibrium approach (range 0.36 ± 0.12 to 3.41 ± 0.74) and using SRTM (range 0.37 ± 0.11 to 3.75 ± 0.75) (Table 1). A comparison of BP_{ND} estimates from equilibrium and SRTM methods for all regions and all scans indicates that they were highly correlated ($r^2 = .95$) across methods for all regions (Figure 3).

To assess the effects of a lack of equilibrium on bias in equilibrium BP_{ND} values, simulations were performed where K_{bol} was varied, for example, under- and over-estimated, and the effect on rates of change between 60 and 90 minutes and bias in BP_{ND} in the putamen was

Table 1. Regional [11C]-(+)-Propyl-Hexahydro-Naphtho-Oxazin Binding Potential (BP_{ND}) Values and Their Test-Retest Characteristics

	Scan 1		Scan 2		Both Scans				
	Mean ± SD	% COV	Mean ± SD	% COV	Mean ± SD	% COV	p Value	% TRV	ICC
BP_{ND} Eq									
Caudate	1.69 ± 0.47	28	1.81 ± 0.40	22	1.75 ± 0.42	24	.64	8.4 ± 9.5	0.94
Putamen	2.49 ± 0.51	21	2.55 ± 0.40	16	2.52 ± 0.44	17	.84	4.9 ± 3.1	0.99
Ventral striatum	3.38 ± 0.80	24	3.45 ± 0.75	22	3.41 ± 0.74	22	.88	−2.6 ± 6.7	0.97
Pallidum	2.85 ± 0.42	15	2.94 ± 0.57	19	2.89 ± 0.48	17	.76	−2.4 ± 8.9	0.92
Substantia nigra	0.94 ± 0.21	23	1.03 ± 0.26	26	0.99 ± 0.23	24	.52	−8.1 ± 21.8	0.72
Thalamus	0.33 ± 0.08	25	0.39 ± 0.14	37	0.36 ± 0.12	32	.40	−12.2 ± 31.9	0.71
BP_{ND} SRTM									
Caudate	1.57 ± 0.41	26	1.72 ± 0.36	21	1.65 ± 0.37	23	.53	9.4 ± 8.9	0.95
Putamen	2.38 ± 0.43	18	2.49 ± 0.33	13	2.44 ± 0.37	15	.62	6.3 ± 5.7	0.96
Ventral striatum	3.62 ± 0.82	23	3.88 ± 0.72	19	3.75 ± 0.75	20	.56	−7.9 ± 10.3	0.92
Pallidum	3.12 ± 0.36	12	3.39 ± 0.44	13	3.26 ± 0.41	13	.27	−8.0 ± 10.3	0.66
Substantia nigra	1.07 ± 0.19	18	1.08 ± 0.35	32	1.08 ± 0.27	25	.98	3.2 ± 28.5	0.60
Thalamus	0.34 ± 0.07	20	0.40 ± 0.14	35	0.37 ± 0.11	30	.33	−2.1 ± 25.7	0.80

COV = coefficient of variation; Eq = equilibrium; ICC = interscan correlation coefficient; SD = standard deviation; SRTM = simplified reference tissue model; TRV = testretest variability.

determined. The results indicate that rates of change up to 10%/h result in a bias in BP_{ND} of ≤ 5% (Figure 4).

Lastly, no age or gender differences in outcome measures were observed.

Discussion

The purpose of this study was to evaluate the feasibility and test–retest reproducibility of striatal and extrastriatal $D_{2/3}$ binding of [11C]-(+)-PHNO using a constant infusion paradigm. The reproducibility of [11C]-(+)-PHNO has been previous explored,[2] but not with a constant infusion paradigm, which has several potential advantages over model-based methods.[10] In our hands, reproducibility of $D_{2/3}$ BP_{ND} using [11C]-(+)-PHNO with both the equilibrium method and

SRTM was excellent, with less than 10% TRV in striatal regions. The two methods were highly correlated (r^2 = .95). Our simulations suggest that a minor lack of equilibrium, that is, rates of change up to 10%/h, produces no more than a 5% bias in BP_{ND}.

The reproducibility of within-subject [11C]-(+)-PHNO binding (bolus injection) has been previously examined with SRTM.[2] Earlier results reported TRV of [11C]-(+)-PHNO BP_{ND} in humans to be 8.7 ± 8% (caudate), 9.9 ± 8% (putamen), 18.6 ± 19% (ventral striatum), and 21.3 ± 16% (pallidum). The TRV in the substantia nigra and thalamus
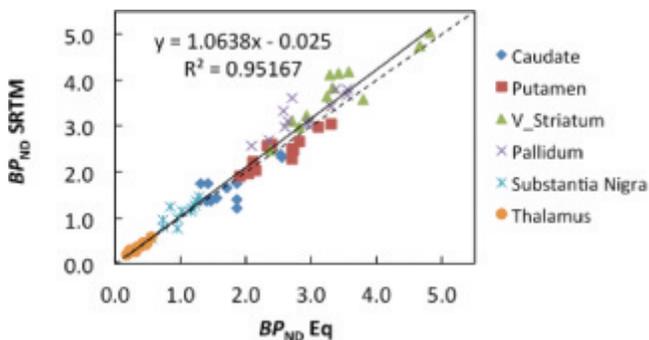
Figure 3. Correlation of binding potential (BP_{ND}) estimates using the equilibrium (60–90 minutes) and simplified reference tissue model (SRTM). The results of the linear regression analysis are given (*solid line*). The *dotted line* is the line of identity.
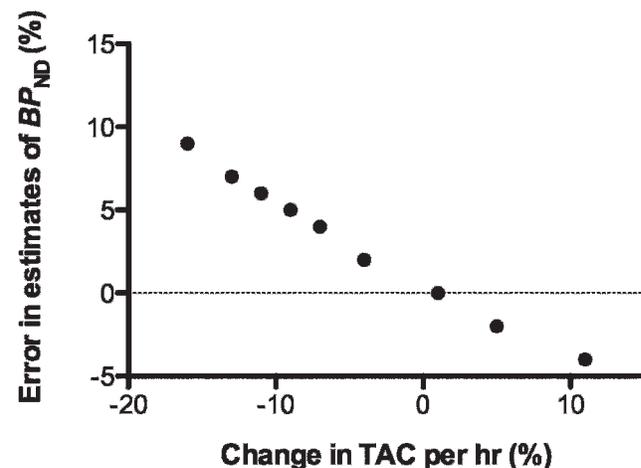
Figure 4. Simulated error in estimated binding potential (BP_{ND}) using the equilibrium method (%) as a function of change in time-activity curve (TAC) per hour (%) determined by simulating over- and underestimates of the infusion parameter K_{bol}.

were not given. In the current study, TRV of BP_{ND} using the SRTM was consistent in the caudate ($9.4 \pm 9\%$) and putamen ($6.3 \pm 6\%$) and better than previously reported in the ventral striatum ($-7.9 \pm 10\%$) and pallidum ($-8 \pm 10\%$). Additionally, in a preliminary study in our laboratory, the TRV in the putamen with the bolus method using SRTM was $9 \pm 6\%$ (Gallezot J-D, unpublished data, 2011). Here we report a modest decrease in TRV in the putamen ($6 \pm 7\%$) using the bolus plus infusion and SRTM.

There are potential advantages to the equilibrium paradigm compared to the more commonly used kinetic modeling techniques to estimate a given outcome measure.[10] One potential advantage is that, in the case of a displacement study, a single-scan, single-radiotracer synthesis and single administration may be sufficient to measure both baseline and stimulus-dependent changes in receptor availability. In the current study, we examined the test–retest reproducibility of $[^{11}C]$-(+)-PHNO given as a constant infusion to lay the groundwork for potential infusion-based displacement studies. In fact, the equilibrium ratio between 45 and 60 minutes was virtually identical in the caudate, putamen, substantia nigra, and thalamus to BP_{ND} values given in Table 1 for the 60- to 90-minute time points, which allows the possibility to perform a displacement at an earlier time point.

A proper bolus to infusion ratio is crucial to obtain accurate outcome measures. Based on regional time-activity curves of previous PHNO bolus injection studies, the optimal bolus to infusion ratio was determined to be $K_{bol} = 80$ minutes. In simulations, this produced equilibrium in the caudate and putamen by 60 minutes of scan start. Using this bolus plus infusion schedule, rates of change $< 8\%/h$ were achieved between 60 and 90 minutes in the caudate, putamen, substantia nigra, and thalamus. Our simulations suggest that variations in time-activity curves up to $10\%/h$ result in small $\leq 5\%$ error in BP_{ND} estimates in the caudate and putamen. This is less than the error associated with TRV. The same infusion ratio ($K_{bol} = 80$ minutes) produced greater change per time ($> 10\%/h$) in the ventral striatum and pallidum and resulted in underestimates of BP_{ND} with the equilibrium method compared to the SRTM (see Table 1). This was predicted by the simulation (see Figure 1), and the error in BP_{ND} estimates in these regions is larger. Thus, for these higher binding regions that require longer time to reach equilibrium, the equilibrium model may result in less accurate estimates of BP_{ND}.

Unfortunately, two of the eight enrolled subjects ended study participation due to nausea and/or emesis. These side effects have been observed by others[9,16] and occurred within 5 minutes of injection. The mass dose (µg/kg) of $[^{11}C]$-(+)-PHNO was not different from what was administered to other subjects. Although a higher total mass (0.05 ± 0.02 µg/kg) injected in the current study than in previous bolus studies (0.03 µg/kg) was suggested to avoid occurrences of side effects,[16] this design was a consequence of the bolus/infusion schedule and is unlikely to account for the side effects. In these two subjects, less than 50% of the total projected mass of radiotracer (0.02 µg/kg) was delivered in the bolus phase, which is in line with the recommended dose (0.03 µg/kg) of $[^{11}C]$-(+)-PHNO, at which there is no correlation between dose and side effects in humans.[16]

Conclusion

$[^{11}C]$-(+)-PHNO PET shows suitable characteristics for $D_{2/3}$ receptor quantification by equilibrium modeling. A bolus plus constant infusion schedule to optimize equilibrium in the caudate nucleus, putamen, and substantia nigra and the requisite equilibrium analysis performs well in comparison with a standard model-based approach. Additionally, equilibrium estimates of BP_{ND} using a constant infusion of $[^{11}C]$-(+)-PHNO were highly reproducible, illustrating the robustness of this paradigm.

Acknowledgment

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