ARTICLE

Occupancy of dopamine D_2 and D_3 receptors by a novel D3 partial agonist BP1.4979: a [^{11}C]-(+)-PHNO PET study in humans

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There has been considerable interest in the development of dopamine D3 receptor (DRD$_3$) partial agonists and antagonists for the treatment of substance use disorders. Pre-clinical evidence overwhelmingly supports the use of these drugs, but translation to humans has remained elusive due to the lack of selective compounds that are suitable for use in humans. Although it has been established for full antagonists, little in vivo occupancy data are available with DRD$_3$ partial agonists. Here we investigate for the first time in healthy controls, the in vivo occupancy of a novel D3 partial agonist (BP1.4979) at the DRD$_3$ and DRD$_2$. Participants received either a single dose (1, 3, 10 or 30 mg) or a subchronic regimen (5–7 days, q.d. or b.i.d) of BP1.4979, with the last dose given at 1, 12 or 24 h prior to scanning with [^{11}C]-(+)-PHNO. Single and subchronic administration of BP1.4979 dose-dependently occupied the DRD$_3$ and DRD$_2$, and this occupancy was preferential for the DRD$_3$, notably at longer time points after administration of BP1.4979. Also consistent with preference for the DRD$_3$, prolactin levels were minimally increased, and no subjective effects of BP1.4979 were reported. Serum levels of BP1.4979 were higher than its active metabolite, BP1.6239, while no notable increases in the inactive metabolite, BP1.6197, were found. These findings indicate the range of doses that can be used to occupy selectively the DRD$_3$ over the DRD$_2$ with BP1.4979 and speak to the use of in vivo imaging approaches in dose finding studies.

INTRODUCTION

Dopamine (DA) has been established as important to substance use disorders [1, 2]. Converging evidence implicates the D$_3$ receptor (DRD$_3$) [3] as a target for medications development [4]. The DRD$_3$ shares homology with the DRD$_2$ [5], and efforts have been focussed on developing ligands that are selective for the DRD$_3$ as compared to the DRD$_2$, especially given that administration of DRD$_2$ antagonists can produce debilitating side effects [6, 7]. A number of compounds selective for the DRD$_3$ have been developed [8] and some antagonists have been tested in humans [9–11]. It is important that novel drugs be evaluated for their occupancy of the DRD$_3$ in vivo, as otherwise the dose range selected for clinical trial studies may be inadequately selected to study the role of DRD$_3$ [12].

PET imaging is a non-invasive technique that allows for the measurement of receptor occupancy. One agonist tracer, [^{11}C]-(+)-PHNO [13], allows for the measurement of occupancy at both DRD$_2$ and DRD$_3$. Through measurement of binding potentials at different regions of interest, regional fractions of DRD$_2$ or DRD$_3$ levels can be determined [12]. In the elegant work of Tziortzi et al.

[14], it was demonstrated that approximately 100% of binding in the substantia nigra (SN) is to the DRD$_3$, 75% of binding in the ventral pallidum (VP) is to the DRD$_3$, while it is 65% in the globus pallidus (GP). These regional fractions are consistent with the animal literature [15, 16] and speak to the validity of using [^{11}C]-(+)-PHNO to measure differentially DRD$_3$ and DRD$_2$.

Imaging with PET and [^{11}C]-(+)-PHNO can provide an important step in the development of DRD$_3$ selective compounds. In our previous study [17], we used this approach to measure occupancy of the DRD$_3$ and DRD$_2$ by buspirone, an antagonist that has been shown, in pre-clinical studies, to have greater affinity for the DRD$_3$ than the DRD$_2$ [18]. In our study, we found that, in humans in vivo, buspirone did not bind more to the DRD$_3$ than the DRD$_2$. This difference in the pre-clinical findings and the clinical data underscores the importance of human in vivo investigations for determination of receptor occupancy levels. Indeed, in a previous study, another antagonist, GSK598809, was administered prior to scanning with [^{11}C]-(+)-PHNO [19]. It was found that GSK598809 displaced [^{11}C]-(+)-PHNO in the SN but not the dorsal caudate (DC), with intermediate displacement in the ventral striatum (VST;

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also known as the limbic striatum (LST)) and GP. However, it remains to be determined whether a DRD$_3$ partial agonist can selectively occupy the DRD$_3$ as compared to the DRD$_2$. In this regard, a recent investigation studied the occupancy of the DRD$_3$ and DRD$_2$ following the administration of cariprazine to participants with schizophrenia. It was found that this DRD$_3$ partial agonist preferentially occupied the DRD$_3$, over the DRD$_2$, at low doses [20].

The purpose of the present study was to investigate, for the first time in healthy controls, the in vivo occupancy of the DRD$_3$ and DRD$_2$ by a selective DRD$_3$ partial agonist. BP1.4979 has an affinity for the human DRD$_3$ of ~1 nM and presents a partial agonist behaviour with an intrinsic activity of 32% ± 2.6% and EC$_{50}$ of 0.7 ± 0.3 nM. In contrast, it behaves as an antagonist at the hDRD$_2$ with K$_i$ of 192 nM. After oral administration in humans, it reaches peak serum concentrations in one hour and has a half-life of about 8 h. In the present study, participants were administered a number of acute doses of BP1.4979 one hour prior to PET scanning with [^{11}C]-(+)-PHNO to characterise the occupancy of DRD$_3$ and DRD$_2$. BP1.4979 was then administered at various time points prior to scanning with [^{11}C]-(+)-PHNO to determine the time course of binding of BP1.4979 to the DRD$_3$ or DRD$_2$. Participants then took BP1.4979 at home for approximately 7 days to determine the effects of sub-chronic dosing of BP1.4979, taken either once or twice a day, on binding to the DRD$_3$ or DRD$_2$. Prolactin and BP1.4979 levels, as well 2 metabolites of BP1.4979 (one pharmacologically active: BP1.6239; and one inactive: BP1.6197) were also measured in serum. Subjective ratings and adverse events were also recorded. It was hypothesised that the percent occupancy of the DRD$_3$ regions would be greater than that for the DRD$_2$ regions following administration of BP1.4979. The aim of this study was to explore the dosing and dosing regimen that optimally produced occupancy of the DRD$_3$ regions above the DRD$_2$ regions.

METHODS
Participants
All procedures were approved by the Centre for Addiction and Mental Health Research Ethics Board and complied with the 1975 Helsinki Declaration (5th revision, 2000). Participants (>19 years old) were recruited from the community and provided written informed consent. Inclusion criteria is provided in Supplementary Material.

Procedure
This single-blind study of BP1.4979 was divided into several parts (sample sizes provided in Table S1 in Supplementary Material):

1. Part 1: Dose-response: The first part was a dose-response study of acute doses (0, 1, 3, 10, 30 mg) that were administered one hour prior to the start of the PET scan. Participants ($n = 6$) received four treatments in fixed order (0, 3, 1 and 10 mg). Three ($n = 3$) participants received placebo followed by 30 mg on separate days.

2. Part 2: Time course: PET scans were started either 12 h after an acute 10 mg dose (10 mg 12 h, $n = 4$), or 24 h after acute doses of either 1, 3 or 10 mg (1 mg 24 h, $n = 2$; 3 mg 24 h, $n = 3$; 10 mg 24 h, $n = 2$). Each participant also underwent a [^{11}C]-(+)-PHNO scan after placebo. Some participants partook in more than one condition.

3. Part 3: Subchronic dosing: Participants took BP1.4979 orally at home once a day for approximately 7 days. In 4 participants, the scan was conducted both 1 and 24 h after the last dose of sub-chronic dose of 10 mg (10 mgSC 1 h, 10 mgSC 24 h). Two other participants were scanned 24 h after the last sub-chronic dose of 3 mg (3 mgSC 24 h). Each

participant also underwent a [^{11}C]-(+)-PHNO scan after placebo. For doses that occurred at home, participants were called at the time of dosing to remind them to take their pill and also to assess compliance.

4. Part 4: B.I.D. dosing: Based on the results of the first 3 parts, it was decided that B.I.D. administration may be preferable to achieve lasting and preferential occupancy of the D3 receptors. Thus, in the fourth part of the study, participants took BP1.4979 orally at home twice a day for 5-7 days at either 5 mg B.I.D., 10 mg B.I.D. or 15 mg B.I.D. ($n = 4$ each group), and were scanned both at 1 h (5mgBID 1 h, 10mgBID 1 h, 15mgBID 1 h) and 12 h (5mgBID 12 h, 10mgBID 12 h, 15mgBID 12 h) after the last dose. Each participant also underwent a [^{11}C]-(+)-PHNO scan after placebo.

To control for absorption of the drug, participants were asked to fast for at least 3 h prior to attending the lab and were given a light snack about 90 min prior to the start of the scan.

During each PET session, 4 blood samples were taken for analysis of serum levels of prolactin, BP1.4979 and metabolites of BP1.4979. The first was taken one hour prior to the start of the scan (to correspond with the time of immediately before dosing). After that, 3 blood samples were taken at one hour intervals. Visual analog scales (VAS; items are reported in the Supplementary Material) were given at baseline (pre-dosing) and at every hour following that. Adverse events were assessed at each visit and for each of the two days following the scans (see Supplementary Material).

Analysis of BP1.4979 and its metabolites (BP1.6239 and BP1.6197)
See Supplementary Material

PET image analysis
Region of interest (ROI)-based analysis. ROI delineation and time activity curve analyses were performed using ROMI (details in ref. [21]). Functional sub-compartments of the striatum [22] including the associative striatum (AST), limbic striatum (LST), and sensor-imotor striatum (SMST) were chosen as ROIs. Delineation for the (whole) VP and SN is described elsewhere [23]. The globus pallidus (GP) was delineated in ROMI.

Binding potential:: [^{11}C]-(+)-PHNO specific binding potential (BP$_{ND}$) was estimated in each ROI using the simplified reference tissue method [24] (SRTM), with cerebellar cortex (excluding vermis) as reference region. Parameter estimation was performed using PMOD (Version 2.8.5; PMOD Technologies Ltd, Zurich, Switzerland).

DRD$_3$ vs. DRD$_2$ component:: As we are interested in the DRD$_3$ vs. DRD$_2$ selectivity of BP1.4979 we estimated the overall average DRD$_3$ component (BPD$_3$') and DRD$_2$ component (BPD$_2$'). To calculate baseline BPD$_3$', BP$_{ND}$ in each ROI (SN, VP, GP, LST, AST, SMST) was multiplied by established regional fractions [14] (fD$_{3(SN)} = 1.0$; fD$_{3(VP)} = 0.75$; fD$_{3(GP)} = 0.65$; fD$_{3(LST)} = 0.26$; fD$_{3(SMST)} = 0.02$; fD$_{3(AST)} = 0$). The product of BP$_{ND}$ and fD$_3$ from each of the 6 ROIs was then averaged to derive BPD$_3$' at baseline. To calculate baseline BPD$_2$', the same equation was applied except that the BP$_{ND}$ of ROIs was multiplied by (1-fD$_3$). Under blocking conditions, BPD$_3$' and BPD$_2$' were the averages of regional BP$_{NDBP1.4979}$[D$_3$] and BP$_{NDBP1.4979}$[D$_2$] calculated with Eqs 4 and 5 (see below), respectively, i.e., using modified fD$_3$ accounting for drug occupancy.

Occupancy: For ROIs, percent occupancy was defined as the percentage reduction in BP$_{ND}$ from 0 mg (placebo) scan to the BP1.4979 exposed state, calculated for each participant using
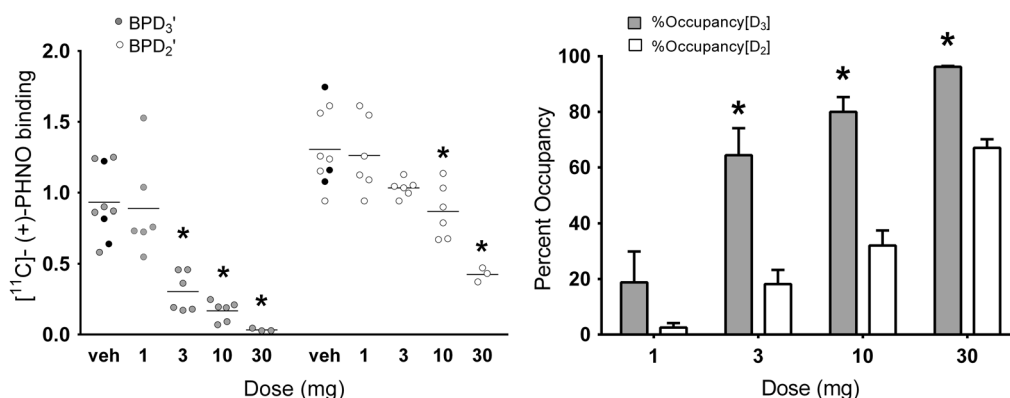
Fig. 1 Dose-response study of acute doses of BP1.4979 administered one hour prior to PET scanning with [^{11}C]-(+)-PHNO. Left panel: BPD$_2$' and BPD$_3$' after administration of acute doses (1, 3, 10 mg: $n = 6$; 30 mg: $n = 3$). Grey and open symbols represent BPD$_3$' and BPD$_2$', respectively; black symbols represent placebo condition for the 30 mg dose group. *$p < 0.05$, different from placebo for either BPD$_2$' or BPD$_3$'. Right panel: Mean ± SEM %Occupacny of the DRD$_3$ (grey sumbols) or DRD$_2$ (open symbols). *$p < 0.05$, %Occupancy[D$_3$] vs. %Occupancy[D$_2$]

the following Eq. 1:

$$\%Occupancy = \left((BP_{ND\,placebo} - BP_{NDBP\,1.4979})/BP_{ND\,placebo} \right) \times 100 \tag{1}$$

To obtain percentage occupancy at DRD$_3$ and DRD$_2$ sites for a blocking scan, assuming constant %Occupancy[D$_3$] and %Occupancy[D$_2$] across all regions, a six-point (6 ROIs) linear regression was performed by using Eq. 2:

$$\%Occupancy = \%Occupancy[D_2] + \\ (\%Occupancy[D_3] - \%Occupancy[D_2]) \times fD_3 \tag{2}$$

This equation was derived from Eqs. 1 and 3–5:

$$BP_{NDBP1.4979} = BP_{NDBP1.4979}[D_3] + BP_{NDBP1.4979}[D_2] \tag{3}$$

$$BP_{NDBP1.4979}[D_3] = (1 - \%Occupancy[D_3]/100) \\ \times BP_{NDplacebo} \times fD_3 \tag{4}$$

$$BP_{NDBP1.4979}[D_2] = (1 - \%Occupancy[D_2]/100) \\ \times BP_{NDplacebo}(1 - fD_3) \tag{5}$$

The model constraints were $0 < \%Occupancy[D_2] < 100$, $0 < \%Occupancy[D_3] < 100$ and $\%Occupancy[D_3] > \%Occupancy[D_2]$, given the observed DRD$_3$ preference of BP1.4979. The linear regressions were performed in GraphPad Prism software.

Data analyses
Differences in DRD$_3$ and DRD$_2$ component were analysed with repeated-measures Dose/Condition × Component (2 levels; BPD$_3$', BPD$_2$') ANOVAs. Significant ANOVAs were followed by Bonferroni-corrected paired t-tests of placebo to each Dose/Condition. % Occupancy was analysed with %Occupancy (2 levels; %Occupancy [D$_3$], %Occupancy[D$_2$]) × Dose/Condition ANOVAs followed by Bonferroni-corrected paired t-tests of %Occupancy[D$_3$] to % Occupancy[D$_2$] for each Dose/Condition. For all analyses, sphericity was assessed with the Mauchley's test and criterion for significance was set at $p < 0.05$. Analyses were conducted with SPSS (version 24).

RESULTS
Participants were 28 non-smokers (20 male; 16 Caucasian, 5 Asian, 2 Hispanic, 2 Mixed, 3 African American). The mean ± SEM

age was $40 ± 2.65$ and all were within normal body mass index ($24.27 ± 0.51$).

Adverse events
BP1.4979 was generally well-tolerated. For a description of adverse events and subjective effects, see Supplementary Material.

See Table S1 (Supplemental Material) for a breakdown of sample sizes in the various conditions.

Part 1: acute dose-response
BPD$_2$' and BPD$_3$' (Fig. 1): Analysis of the differences in BPD$_3$' and BPD$_2$' component with a repeated-measures Component (2 levels; BPD$_2$', BPD$_3$') × Dose (4 levels; 0, 1, 3, 10; the 30 mg dose is not included in the ANOVA because it consisted of a different group of participants) ANOVA revealed a significant interaction ($F(3,15) = 8.454$, $p = 0.002$; $n = 6$), indicating that the effect of BP1.4979 dose-dependently decreases [^{11}C]-(+)-PHNO binding differently in the BPD$_3$' and the BPD$_2$' (Fig. 1). Bonferroni-corrected t-tests found that, for the BPD$_3$', placebo was different from the 3 and 10 mg doses, and for the BPD$_2$', placebo was different from the 10 mg dose ($n = 6$; adjusted p value of 0.0167). In a separate ANOVA for the 30 mg dose ($n = 3$), the Component (2 levels; BPD$_2$', BPD$_3$') × Dose (2 levels; 0, 30) interaction was not significant ($p > 0.1$), but there was a significant effect of Dose ($F(1, 2) = 25.721$. $p = 0.037$) and Component ($F(1,2) = 55.859$, $p < 0.017$), suggesting that BP1.4979 decreased BPD$_2$' and BPD$_3$' to the same extent. t-Tests on the difference between placebo and the 30 mg dose, separately for each of the BPD$_3$' and BPD$_2$' revealed significant effects for both of these comparisons ($p < 0.05$).

%Occupancy: Analysis of %Occupancy with a %Occupancy (2 levels; %Occupancy[D$_3$], %Occupancy[D$_2$]) × Dose (3 levels; 1, 3, 10 mg) ANOVA on the dose-response revealed a significant interaction ($F(2, 10) = 9.427$, $p = 0.005$), indicating that the effect of Dose is different for %Occupancy[D$_3$] or %Occupancy[D$_2$]. Bonferroni-corrected t-tests revealed that %Occupancy[D$_3$] is different from %Occupancy[D$_2$] at the 3 and 10 mg doses (adjusted p value of 0.0167). Separate Bonferroni-corrected t-tests on the 30 mg dose revealed that %Occupancy[D$_3$] and % Occupancy[D$_2$] were different ($p < 0.05$).

Part 2: time course analysis
BPD$_2$' and BPD$_3$' (Fig. 2): Analysis of the differences in BPD$_3$' and BPD$_2$' component with repeated-measures Component (2 levels; BPD$_2$', BPD$_3$') × Condition (2 levels; placebo vs. 3 mg 24 h or 10 mg 12 h) ANOVAs revealed a significant interaction for 10 mg 12 h condition ($F(1, 3) = 30.925$, $p = 0.011$), and only an effect of Component for the 3 mg 24 h Condition ($n = 3$; F1, 2) = 19.773, $p = 0.047$). Bonferroni-corrected paired t-tests suggested that the
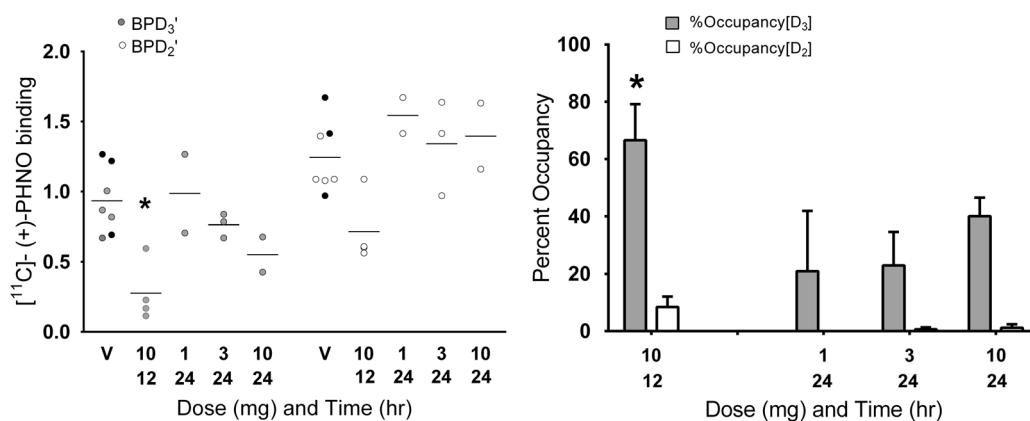
Fig. 2 Time course study of several acute doses (1, 3 and 10 mg) of BP1.4979 administered at various times (12 or 24 h) prior to PET scanning with [^{11}C]-(+)-PHNO. Left panel: BPD_2' and BPD_3' after administration of acute doses (10 mg 12 h, $n = 4$; 10 mg 24 h, $n = 2$; 1 mg 24 h, $n = 2$; 3 mg 24 h $n = 3$). Grey and open symbols represent BPD_3' and BPD_2', respectively. Black symbols represent the placebo condition for 3 mg 24 h group. Placebo conditions not shown for 1 mg 24 h and 10 mg 24 h conditions because the small sample sizes precluded analyses. *Different from placebo for the BPD_3' ($p < 0.05$). Right panel: Mean ± SEM percent occupancy of the DRD_3 (grey sumbols) or DRD_2 (open symbols). *$p < 0.05$, %Occupancy[D_3] vs. %Occupancy[D_2]

10 mg 12 h ($n = 4$) Condition was significantly different from placebo for the BPD_3' ($p = 0.023$; adjusted p value of 0.025) but not the BPD_2' ($p = 0.132$; adjusted p value of 0.025); no significant effects for the 3 mg 24 h Condition were revealed by Bonferroni-corrected t-tests. For the 1 mg 24 h and 10 mg 24 h Conditions, the small sample sizes ($n = 2$) precluded statistical analyses. Data is provided for visual inspection in Fig. 2.

%Occupancy: Analysis of occupancy with ANOVAs was precluded due to the single condition in the 3 mg 24 h or 10 mg 12 h groups, but Bonferroni-corrected t-tests revealed that the % Occupancy[D_2] was different than the %Occupancy[D_3] at the 10 mg 12 h condition, but not the 3 mg 24 h condition (adjusted p value of 0.025). For the 1 mg 24 h and 10 mg 24 h Conditions, the small sample sizes ($n = 2$) precluded statistical analyses. Data is provided for visual inspection in Fig. 2.

Part 3: sub-chronic dosing
BPD_2' and BPD_3' (Fig. 3): Analysis of the differences in BPD_3' and BPD_2' component with a repeated-measures Component (2 levels; BPD_2', BPD_3') × Condition (3 levels; placebo, 10 mgSC 1 h, 10 mgSC 24 h) ANOVA revealed a significant interaction (F(2, 6) = 5.696, $p = 0.041$, $n = 4$). Bonferroni-corrected t-tests of each Condition to placebo revealed that placebo was different from 10 mgSC 1 h, but not 10 mgSC 24 h, for fD_3' and fD_2' (adjusted p value of 0.025). For the 3 mgSC 24 h Condition, the small sample size ($n = 2$) precluded analysis, but data is provided in Fig. 3 for visual inspection.

%Occupancy: Analysis of occupancy with a %Occupancy (2 levels; %Occupancy[D_2], %Occupancy[D_3]) × Condition (2 levels; 10 mgSC 1 h, 10 mgSC 24 h) ANOVA did not reveal an interaction, but an effect of %Occupancy was found (F(1, 3) = 42.274, $p = 0.007$), suggesting that %Occupancy[D_2] and %Occupancy[D_3] were different. Bonferroni-corrected t-tests revealed that % Occupancy[D_2] was different from %Occupancy[D_3] for the 10 mgSC 1 h condition (adjusted p value of 0.025), but not the 10 mgSC 24 h condition. Analysis of the 3 mgSC 24 h condition was precluded due to the small sample size. Data are presented for visual inspection.

Part 4: B.I.D. administration
BPD_2' and BPD_3' (Fig. 4): Analysis of the differences in BPD_3' and BPD_2' component with a mixed Component (2 levels; BPD_2', BPD_3') × Condition (3 levels; placebo, 1, 12 h) × Dose (3 levels; 5mgBID, 10mgBID, 15mgBID) ANOVA with Dose as the between-subjects

factor revealed a three-way interaction (F(4, 18) = 10.853, $p = <0.001$; $n = 4$ each group), suggesting that the effects of BP1.4979 were different in BPD_2' and BPD_3' at the various time points after treatment and that this varied by dose. Follow-up analysis with Bonferroni-corrected t-tests revealed that the 1 h condition was different from placebo for each of the BPD_2' and the BPD_3' for all doses (adjusted p value of 0.0125). Placebo was also different from the 12 h condition for BPD_3' for the 10mgBID and 15mgBID doses (adjusted p value of 0.0125). For the BPD_2', the 12 h condition was different from placebo for the 10mgBID dose (adjusted p value of 0.0125).

%Occupancy. Analysis of occupancy with a %Occupancy (% Occupancy[D_2], %Occupancy[D_3]) × Condition (1, 12 h) ANOVA separately for each of the 5, 10 or 15 mg doses revealed an effect of %Occupancy (F(1, 3) = 14.748, $p = 0.031$) and Condition (F(1, 3) = 24.289, $p = 0.016$) for the 5 mg dose and also effects of % Occupancy (F(1, 3) = 169.858, $p = 0.001$) and Condition (1, 3) = 49.820, $p = 0.006$) for the 10 mg dose. For the 15 mg dose, a significant interaction was revealed (F(1, 3) = 14.860, $p = 0.031$). This indicates that %Occupancy[D3] was greater than %Occupancy[D2] for the 5 and 10 mg doses (with Occupancy being greater in the 1 h as compared to the 12 h condition), and that the condition had an effect on this difference for the 15 mg dose. Bonferroni-corrected t-tests revealed that %Occupancy[D_2] was different from %Occupancy[D_3] at all conditions and doses, except when participants were scanned 12 h after the last dose of 5 mg B. I.D. (adjusted p value of 0.025)

%Occupancies for various ROIs are provided in Supplementary Material, Table S2.

Scan parameters are provided in Supplementary Material, Table S3.

Analyses of prolactin, BP1.4979, BP1.639 and BP1.6197 are provided in Supplementary Material (Tables S4-S7).

DISCUSSION
The purpose of the present study was to investigate, for the first time in healthy participants, the binding of a DRD_3 partial agonist to DRD_2 and DRD_3. It was found, with PET imaging with [^{11}C]-(+)-PHNO, that BP1.4979 occupied the DRD_3 more than the DRD_2, consistent with a previous report [20]. Compared to control conditions, [^{11}C]-(+)-PHNO binding was decreased when participants were scanned 1 h after administration of BP1.4979; at 1 h
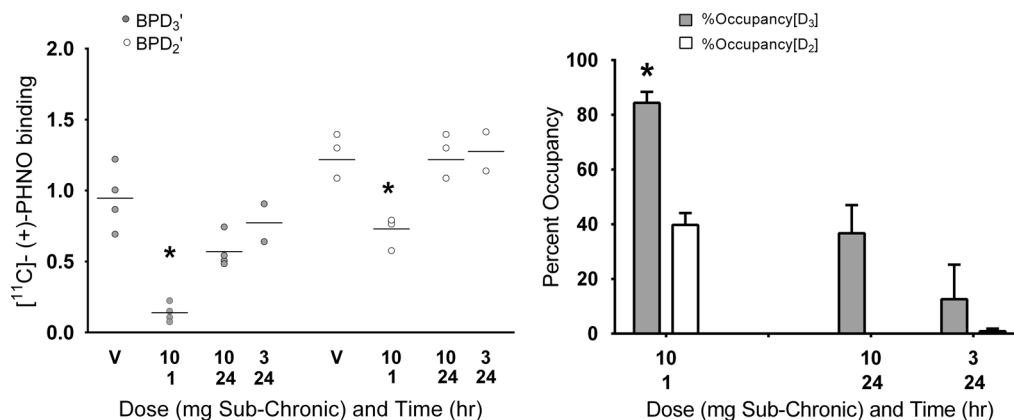
Fig. 3 Study of once daily subchronic doses of BP1.4979, with the last dose administered one hour or 24 h prior to PET scanning with [^{11}C]-(+)-PHNO. Left panel: BPD$_2$' and BPD$_3$' after administration of the acute doses (10 mgSC 1 h, $n = 4$; 10 mgSC 24 h, $n = 4$; 3 mgSC 24 h, $n = 2$). Grey and open symbols represent BPD$_3$' and BPD$_2$', respectively. Placebo not shown for 3 mg 24 h because the small sample size precluded analyses. *$p < 0.05$, different from placebo for either BPD$_2$' or BPD$_3$'. Right panel: Mean ± SEM percent occupancy of the DRD$_3$ (grey sumbols) or DRD$_2$ (open symbols). *$p < 0.05$, %Occupancy[D$_3$] vs. %Occupancy[D$_2$]

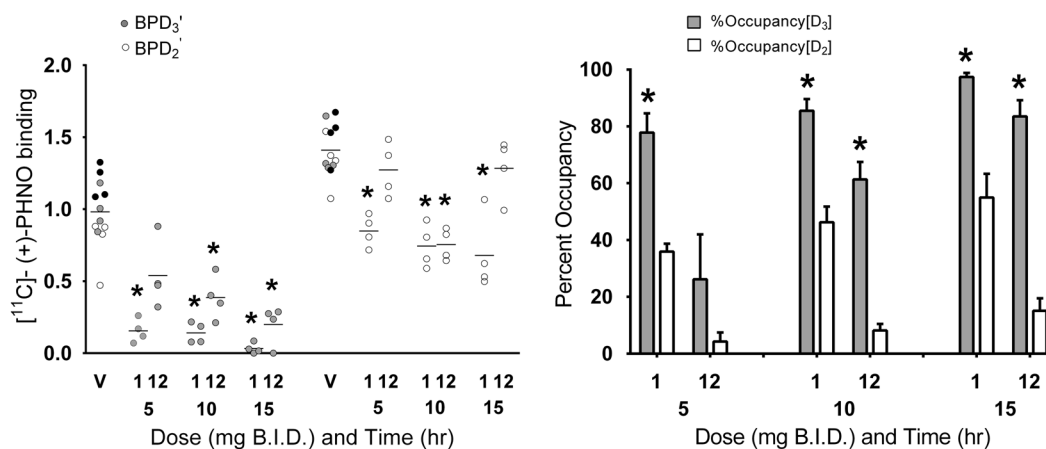**Fig. 4** Study of B.I.D. (twice daily) subchronic doses of BP1.4979, with the last dose administered one hour or 12 h prior to PET scanning with [^{11}C]-(+)-PHNO. Left panel: BPD$_2$' and BPD$_3$' after administration of the acute doses ($n = 4$ for each dose). Grey and open symbols represent BPD$_3$' and BPD$_2$', respectively. Open, grey and dark symbols at placebo conditions represent the 5, 10 and 15 mg doses, respectively. *$p < 0.05$, different from placebo for either BPD$_2$' or BPD$_3$'. Right panel: Mean ± SEM percent occupacny of the DRD$_3$ (grey sumbols) or DRD$_2$ (open symbols). *$p < 0.05$, %Occupancy[D$_3$] vs. %Occupancy[D$_2$]

after administration of BP1.4979, occupancy of the DRD$_3$ was higher than the DRD$_2$. When scanned at various times after administration of BP1.4979, there was residual occupancy of the DRD$_3$ at 12 h post-dose, most notably following B.I.D. administration. Also after 12 h, [^{11}C]-(+)-PHNO binding was decreased more at the DRD$_3$. Inspection of occupancy data suggests that BP1.4979 may occupy the DRD$_3$ more than the DRD$_2$ when participants were scanned 24 h after being given BP1.4979. Changes in [^{11}C]-(+)-PHNO binding and percent occupancy showed region-dependent changes, with greater effects of BP1.4979 in areas with higher fD$_3$. Change in [^{11}C]-(+)-PHNO binding and percent occupancy were more long-lasting than measured increases of serum levels of BP1.4979 or its active metabolite, BP1.6239, and this was most evident after B.I.D. administration. There were small, non-significant increases in prolactin at most doses tested. There was no change in subjective ratings following administration of BP1.4979. BP1.4979 was well-tolerated.

In the present study, dose-dependent decreases in [^{11}C]-(+)-PHNO binding in the BPD$_3$' and BPD$_2$' were observed. These decreases were significant for the BPD$_3$' when 3, 10 and 30 mg were administered one hour prior to scanning; for the BPD$_2$', these

changes were significat after 10 and 30 mg doses. When single acute doses were administered 12 or 24 h prior to PET scanning with [^{11}C]-(+)-PHNO, significant decreases were observed only in only the BPD$_3$' 12 h after administration of BP1.4979. When 10 mg was administered subchronically once daily, BPD$_2$' and BPD$_3$' were decreased at one hour after the last dose, but not at 24 h, but residual occupancy was noted for the DRD$_3$. By contrast, B.I.D. administration produced long-lasting changes in regional fraction that were evident at 12 h after the last dose following 10 and 15 mg B.I.D. administration in BPD$_3$' and BPD$_2$'; occupancies were greater in BPD$_3$' 12 h after dosing. Thus, administration of BP1.4979 at 12 h intervals seems to produce the most long-lasting changes at the DRD$_3$ (and DRD$_2$).

Even though [^{11}C]-(+)-PHNO binding was decreased in both the BPD$_2$' and BPD$_3$', the percent occupancy of the DRD$_3$ was higher than that for the DRD$_2$ following administration of BP1.4979. Differences in occupancy of the DRD$_3$ and DRD$_2$ were observed at all doses, but the selectivity was greatest below 30 mg and above 1 mg, with the best relative occupancy being observed at 10 mg, determined by the magnitude of separation between DRD$_2$ and DRD$_3$ separation (BPD$_3$': 80%; BPD$_2$': 32%). It should be noted that

it is not clear if there is any therapeutic benefit to this separation in occupancy. Time course analysis further revealed that occupancy of the DRD$_3$ was more long-lasting than for the DRD$_2$, with the 24 h pre-treatment time being associated with the best lasting occupancy of the DRD$_3$ (40%; DRD$_2$: 1%). Interesting effects were seen, however, during B.I.D. dosing. Under this regimen, persistent occupancy of the DRD$_3$ was seen 12 h after the last dose (10 mg B.I.D: 61%; 15 mg BID: 83%), where this was not observed to nearly the same extent for the DRD$_2$ (10 mg B.I.D: 8%; 15 mg B.I.D.: 15%). Thus, it appears that 10 mg B.I.D. and 15 mg B.I.D. seem to be the best dosing regimens for achieving lasting occupancy of the DRD$_3$ as compared to the DRD$_2$.

The DRD$_2$ an DRD$_3$ component approach allows for the quantification of the amount of occupancy of the DRD$_3$ relative to the DRD$_2$. Inspection of the changes in relative occupancies in various ROIs provides validation for this approach. The degree of change in occupancy in the various ROIs was greatest in the D3-rich area of the SN, where approximately 100% of the signal is due to DRD$_3$, while the change in regional fraction became progressively smaller in ROIs with more of a D2 signal (AST, SMST). These findings suggest that BP1.4979 is more potent in the D3-rich areas and also occupies the DRD$_3$ more selectively than the DRD$_2$.

Prolactin is released by the pituitary gland and is under inhibitory control of DA. Blockade of the DRD$_2$ releases this inhibition. Increased prolactin (hyperprolactinemia) is a known side effect of treatment with agents that block the DRD$_2$, and thus prolactin levels provide a general estimate of the efficacy of a treatment in blocking the DRD$_2$. In a review of studies on the effects of antipsychotics on prolactin levels, consensus values for estimates of hyperprolactinemia were in the range of about 20-30 ng/ml [25]. Thus, in the present study, good selectivity at the DRD$_3$ is supported by the further finding that prolactin levels were not greatly affected by any dose or pre-treatment regimen with the exception of the highest doses of the acute 30 mg dose and the sub-chronic 15 mg B.I.D. dosing.

Selective effects of BP1.4979 on the DRD$_3$ as opposed to the DRD$_2$ are consistent with the pre-clinical literature that the debilitating side effects of treatments with dopamine antagonists are related to actions at the DRD$_2$ [12]. Indeed, in the present study, BP1.4979 was well-tolerated. This is consistent with the pre-clinical evidence that the effects of D3 ligands on behaviour are fairly selective and devoid of any off-target events [26]. It should be noted that, in the present study, participants were also undergoing scanning procedures and thus there is some ambiguity as to whether some adverse events recorded were due to the scanning procedure. Regardless, ratings on the VAS were not affected and all adverse events were mild in intensity.

BP1.4979 has two main metabolites: BP1.6239 and BP1.6197, the former being an active metabolite. In the present study, no appreciable increases in BP1.6197 were observed. By comparison, levels of BP1.4979 and BP1.6239 were increased in a manner that is consistent with their known pharmacokinetics (half life of 8 h and reaching peak values within one hour). What was surprising is that the occupancy of the DRD$_3$ was more long-lasting than the elevations in serum levels of BP1.4979. This is most apparent in consideration of the B.I.D. dosing regimen, where BP1.4979 and BP1.6239 were negligible at 12 h after the last dosing under 10 mg B.I.D. or 15 mg B.I.D., but the occupancy of the DRD$_3$ remained at 61% and 83%, respectively. This suggests that the elimination of the drug from the brain has different pharmacokinetics than it does systemically. This observation warrants further exploration and speaks to the importance of using in vivo PET imaging in dose finding studies, especially for drugs that are believed to have psychotropic effects.

In summary, BP1.4979, and DRD$_3$ partial agonists, warrant further exploration as treatment approaches that target the DRD$_3$. Based on the results of the present study, a B.I.D. dosing regimen may be optimal for achieveing long-lasting occupancy of the DRD$_3$, even after it has cleared from the plasma. More specifically for BP1.4979, it appears that 10 mg B.I.D. or 15 mg B.I.D. may have the best persistent occupancy of the DRD$_3$. However, 15 mg B.I.D. also increased prolactin levels, and thus 10 mg B.I.D. may be the optimal dose for further testing.

LIMITATIONS
This study is not without limitations. Briefly: (1) The sample size in this study was small. This limits generalisability of the results somewhat and may also explain the lack of power in some of the analyses. Despite this, effects were quite apparent and the interpretation of the results would likely not be changed with a larger sample size; (2) This study is underpowered to observe effects of gender [27]; (3) The total (combined labelled and unlabelled) mass injected was lower for the 10 mg dose as compared to placebo in the acute dose study. However, the direction of change in effect is not the same as that which would be predicted by the difference in mass. Thus, the differences in mass injected likely did not have any effect in the present study. This is supported by the further findings that the 10 mg dose produced comparable changes in regional fraction in other parts of this study where the mass injected was not different from placebo; and (4) SRTM is known to underestimate BP$_{ND}$ in regions of high binding [28]. However, this effect seems to be negligible as shown in previous [^{11}C]-(+)-PHNO PET occupancy studies (Girgis et al. [19, 29]).

CONCLUSIONS
The present study is the first to investigate, in healthy participants, the in vivo occupancy of DRD$_2$ or DRD$_3$ by a DRD$_3$ partial agonist. BP1.4979 had greater occupancy of DRD$_3$ as opposed to DRD$_2$, consistent with findings that prolactin levels were not significantly affected at most doses and that subjective ratings were not altered at any dose. These findings are consistent with converging pre-clinical evidence that targeting the DRD$_3$, as opposed to the DRD$_2$, can provide a therapeutic target for the development of treatments for substance dependence. They also point to a critical role of in vivo occupancy in dose finding for new therapeutic targets.

AUTHOR CONTRIBUTIONS:
PDC oversaw the day-to-day conduct of the study, analysed the data and wrote the first draft of the manuscript. EM conducted part of the study and PET analyses. JT assisted with the data analyses and interpretation, AAW oversaw the radioligand synthesis, SH oversaw the PET imaging, IB provided oversight for imaging analysis and contributed to interpretation of the data results. TD provided assistance with regulatory issues and drug related issues and study design, PR oversaw analyses of BP1.4979 and metabolites. JCS provided designed the study and interpretation of the data. BLF provided oversight of all study component as Principal Investigator and Qualified investigator. He designed the study. All authors participated in manuscript writing. J-C.S. is co-founder, shareholder and Scientific manager at Bioprojet, B.L.F. received funding from Bioprojet for this project.

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ADDITIONAL INFORMATION

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