Protocol Title: Evaluation of a novel PET radioligand for phosphodiesterase-4D (PDE4D)
Abbreviated Title: PET imaging of PDE4D
Protocol Number: 19-M-0064
Date of This Submission/Version: 4/3/19

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Human Research Protections Program Investigator and Staff Training:

For this protocol, the following "just in time" human subjects protection training courses are required for investigators and staff:

- NIAID GCP course or CITI GCP modules
- Unanticipated Problems and Reporting Requirements in Biomedical Research

Total requested accrual
(0) Patients

(40) Healthy Volunteers

Project Uses Ionizing Radiation: □ No ■ Yes (*attach RSC/RDRC documentation)*
□□Medically-indicated only
■□Research-related only
□□Both

IND/IDE □ No ■ Yes *(attach FDA documentation)*
Drug: ^{11}C-T1650. Not yet submitted
Sponsor: NIMH IRP

Drug: BPN14770: #127,905
Sponsor: Tetra Discovery Partners, Grand Rapids, MI

Durable Power of Attorney ■ No □ Yes

Multi-institutional Project ■ No □ Yes
Institution#1_____ FWA #_____
Date of IRB approval _____*(attach IRB documentation)*

Institution#2_____ FWA #_____
Date of IRB approval _____*(attach IRB documentation)*

Data and Safety Monitoring Board □ No ■ Yes

Technology Transfer Agreement □No ■ Yes
Agreement type and number _CTA_____ Expiration Date_01/27/2028_____

Samples are being stored ■ No □ Yes
Covered protocol requiring DEC approval □No ■ Yes
Flesch-Kincaid reading level of consent forms:

- Phase 1: 8.9
- Phase 2: 8.7
- Phase 3: 8.9
- Phase 4: 8.7

Précis:

i. Objective

Phosphodiesterase type 4 (PDE4) metabolizes 3′,5′-cyclic adenosine monophosphate (cAMP), thereby terminating this second messenger. PDE4 is selective to cAMP over cyclic guanosine monophosphate. PDE4 has four isozymes—A, B, C, and D—and basic studies suggest that type D (PDE4D) may play a key role in cognitive function and depression. That is, PDE4D inhibitors are expected to improve cognitive function and depressive symptoms.

In collaboration with Tetra Discovery Partners, we have developed a PET ligand, ^{11}C-T-1650, to selectively image PDE4D. This type D selective ligand was developed based on 3D structural differences between PDE4D and PDE4 type B (PDE4B) Our PET studies using ^{11}C-T-1650 in nonhuman primate have shown promising results, and we now seek to evaluate it in in healthy subjects.

This study has three primary objectives. First, we will determine whether the uptake of ^{11}C-T-1650 in the brain reflects the distribution of PDE4D, as demonstrated by blocking with a PDE4D selective compound BPN14770, being developed by Tetra Discovery Partners for treating cognitive disorders including depression. Second, we will measure binding site occupancy of BPN14770 administered at doses that may be used in clinical trials. Third, we will measure the test/retest reproducibility of brain uptake quantified by kinetic modeling and using arterial blood samples.

ii. Study Population

Healthy adult female and male volunteers (age ≥ 18) will have either brain (n = 30) or whole body imaging (n = 10).

iii. Design

a) Phase 1: We will begin with whole body scanning in a single human subject using up to 10 mCi ^{11}C-T-1650 The aim of this first scan will be to detect a tracer that disproportionately accumulates in a single radiosensitive organ, such as the gonads. If we confirm that radioactivity is fairly widely distributed in the body, higher activities may be injected.

b) Phase 2: Fifteen healthy subjects will have three brain PET scans using 20 mCi of ^{11}C-T-1650. Scan 1 will serve as the baseline scan for comparison to enzyme occupancy studies (Scan 2 and 3). Scans 2 and 3 will be enzyme occupancy studies using the PDE4D selective medication BPN14770.Scans 2 and Scan 3 will be performed approximately 90-180 min after the first dose of BPN14770 and after three-day administration, respectively. Scan 3 will be performed approximately 90-180 min after the last dose of the three-day administration of BPN14770. The dose of BPN14770 is 50 mg BID for three days, and a single dose on the fourth day. Comparison between Scan 1 and 2 provides accurate measurement of nonspecific binding of ^{11}C-T-1650. Comparison between Scan 1 and 3 provides enzyme occupancy at a stable plasma concentration of BPN14770 based on Phase 1/2 clinical trials performed by Tetra Discovery Partners. Blood samples will be measured for BPN14770 levels.

c) Phase 3: To obtain dosimetry information, we will perform a whole body PET scan using ^{11}C-T-1650 (20 mCi) in up to nine healthy subjects.

d) Phase 4: Fifteen healthy subjects will have two brain PET scans using 20 mCi of ^{11}C-T-1650 to study test/retest reproducibility of the PET measurement. BPN14770 will not be administered in Phases 1, 3 or 4.

iv. Outcome Measures

For whole body imaging, organ uptake will be quantified as a Standardized Uptake Value (SUV), which normalizes for injected activity and body weight. For Scan 1 and 2 of Phase 2 and all scans of Phase 4, uptake will be quantified as total distribution volume (V_T) calculated with kinetic modeling and serial concentrations of parent radioligand in arterial plasma. For Scan 3 of Phase 3, brain uptake (SUV) normalized to plasma concentrations of ^{11}C-T-1650 will be used to measure radioligand binding because the interval between Scan 2 and 3 is too short to repeat placing arterial line. From V_T or normalized SUV, occupancy of BPN14770 and nonspecific binding of ^{11}C-T-1650 will be measured. Occupancy of PDE4D will be compared to blood levels of BPN14770.

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List of Abbreviations

AUC	area under the curve
BID	twice a day
BP_{ND}	Binding potential relative to nondisplaceable distribution volume, specific-to-nondisplaceable ratio of distribution volume
cAMP	3′,5′-cyclic adenosine monophosphate
MRI	magnetic resonance imaging
PDE4	phosphodiesterase type 4
PDE4D	PDE4 subtype D

PET	positron emission tomography
SUV	standardized uptake value
BP_{ND}	specific-to-nondisplaceable ratio of distribution volume
V_T	total (specific plus nondisplaceable) distribution volume

1. Introduction and Background

PDE4D inhibitors produce cognitive improvement and may show antidepressant effects

PDE4 terminates signal transduction of G-protein-coupled receptors by metabolizing the second messenger cAMP. Because of the prevalence of G-protein-coupled receptors, PDE4 plays critical roles in a number of brain disorders including Alzheimer's disease and major depressive disorder.

A large number of pre-clinical and clinical trials have been performed using PDE4 inhibitors. Uncontrolled clinical studies conducted in the late 1980s and early 1990s with rolipram, an isozyme nonselective PDE4 inhibitor, found that this agent had antidepressant effects; however, the ratio of efficacy to adverse reactions limited its clinical use [1]. Two possible causes have been hypothesized for the low ratio of efficacy to adverse reaction: little selectivity of rolipram among the four PDE4 isozymes [2] and high inhibition of PDE4 activity because rolipram is a competitive inhibitor [3]. Building on these hypotheses, PDE4D selective negative allosteric modulators have been developed which induced cognitive improvement without causing emesis [3]. Inhibition of PDE4D is also expected to show antidepressant effects because PDE4D KO mouse showed antidepressant-like effect on behavior [4]

As described in the following subsections in detail, we have performed extensive studies using ^{11}C-(*R*)-rolipram, which binds to all four PDE4 isozymes. To further investigate the role of PDE4 in brain disorders, a PET ligand that binds selectively to one of the four PDE4 isozymes is needed. Tetra Discovery Partners has developed a new PDE4D selective ligand, ^{11}C-T-1650. We tested ^{11}C-T-1650 in monkey PET studies and confirmed that specific binding of ^{11}C-T-1650 is greater than that of ^{11}C-(*R*)-rolipram, although T-1650 binds to only one subtype of PDE4 while rolipram binds to all subtypes. This specific binding suggests that ^{11}C-T-1650 has much higher in vivo affinity than ^{11}C-(*R*)-rolipram for PDE4.

We will also measure the enzyme occupancy by doses of BPN14770 that might be used in clinical studies and relate this to plasma levels of the drug. If all studies proceed well, the results will be used to guide a therapeutic trial in major depressive disorder (MDD) to improve cognitive function and depressive symptoms.

Previous PET studies using ^{11}C-(*R*)-rolipram, a PDE4 isozyme nonselective inhibitor

PET imaging of the cAMP cascade

PET imaging of PDE4 with ^{11}C-(*R*)-rolipram has been successfully used to study cAMP cascade activity *in vivo*. PDEs hydrolyze the second messengers cAMP and cyclic guanosine monophosphate (cGMP) to terminate signal transduction. Eleven PDEs exist in the human body. PDE4 is selective to cAMP and is present in both brain and peripheral organs such as heart, lungs, kidney [5], immune cells [6], osteoclasts [7], and osteoblasts [8]. Rolipram is a selective PDE4 inhibitor; PDE4 has four isozymes, and rolipram is not selective among these isozymes.

Our studies—described below—demonstrated that ^{11}C-(*R*)-rolipram PET is a valuable method for detecting cAMP cascade activity in living human and animal subjects. Because PDE4 is present in almost all brain regions and no regions without PDE4 are available to use as a reference to measure rolipram binding, we used ^{11}C-(*R*)-rolipram concentrations in arterial plasma (i.e., metabolite-corrected arterial input function) to measure rolipram binding in the brain [9, 10]. We also showed that rolipram binding can be accurately measured using only four arterial samples [9].

Broadly, the cAMP theory of depression posits that depression is caused by low cAMP signaling. The corresponding theory regarding the relevant mechanism of treatment is that chronic, but not acute, administration of antidepressants upregulates cAMP signaling. Although the cAMP theory of depression has limited supporting data, evidence for the mechanism of antidepressants has repeatedly been confirmed in animal studies. Chronic, but not acute, administration of all classes of antidepressants, as well as electroconvulsive therapy, upregulates several components of the cAMP pathway, including PDE4.

PDE4, an important component of the cAMP cascade, selectively metabolizes cAMP in the brain to the inactive monophosphate. Rolipram is a reversible inhibitor of PDE4, and binding of ^{11}C-(*R*)-rolipram provides a measure of the activity of this enzyme in the brain. Due to a feedback mechanism, *in vivo* binding of ^{11}C-(*R*)-rolipram reflects the activity of the cAMP cascade; essentially, increased cAMP stimulates protein kinase A (PKA), which phosphorylates PDE4 that, in turn, increases rolipram binding.

We confirmed in animals that increased ^{11}C-(*R*)-rolipram binding reflects the phosphorylated / active state of PDE4. Using this radioligand, we found that PDE4 binding is decreased in unmedicated patients with MDD, consistent with the cAMP theory of depression. Finally, we found that two months of treatment with an SSRI increased (normalized) PDE4 binding, consistent with the cAMP theory of the mechanism of antidepressants.

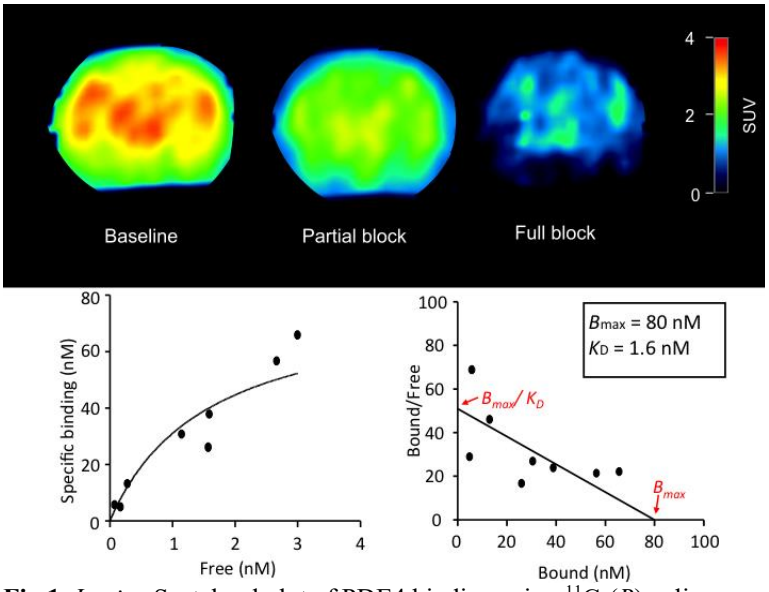

Fig 1. *In vivo* Scatchard plot of PDE4 binding using ^{11}C-(*R*)-rolipram in awake rats. PET scans were performed with increasing concentrations of radioligand in order to saturate binding to PDE4 (bottom left), and analyzed as an *in vivo* Scatchard plot (bottom right). The brains were subsequently harvested for *in vitro* Scatchard analysis. Binding density (Bmax) was unchanged, but binding affinity was decreased five- to six-fold after death compared to the awake condition. The decreased affinity after death is consistent with the rapid dephosphorylation (within minutes) of PDE4. The phosphorylation state of PDE4 would not be possible in human postmortem tissue; PET imaging is the only technique currently available to measure the phosphorylated and active state of this enzyme.

In Vivo Density and Affinity. Our initial experiments sought to measure *in vivo* both binding site density and radioligand affinity of ^{11}C-(*R*)-rolipram in the rat brain [11]. We also studied two critical factors in small-animal PET scans: the influence of anesthesia and the difference in binding under *in vivo* and *in vitro* conditions. Binding site density and radioligand affinity of conscious rats were significantly greater than those of anesthetized rats, by 29% and 59%, respectively. In addition, *in vitro* affinity was five-fold greater than *in vivo* affinity, although density was similar in both conditions (Fig 1). The findings were consistent with rapid dephosphorylation of PDE4 and established that ^{11}C-(*R*)-rolipram binding *in vivo* reflects cAMP cascade activity in rats.

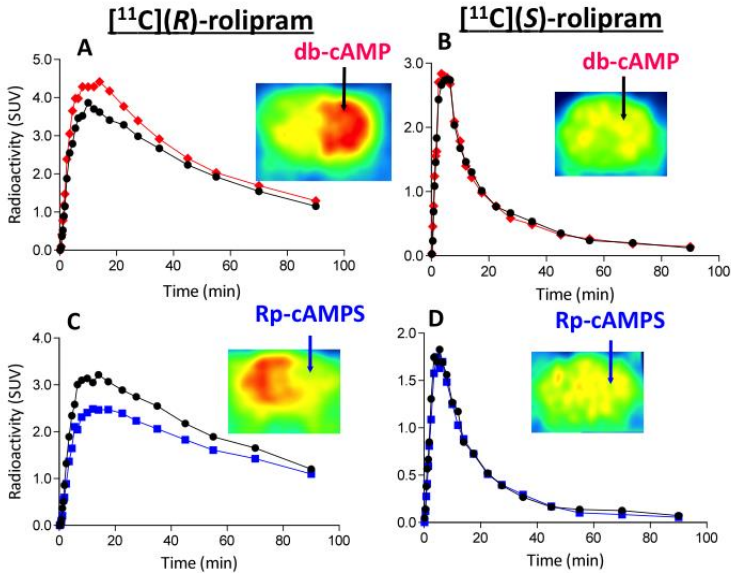

Fig 2. ^{11}C-(*R*)-rolipram binding in rat brain depends on the phosphorylation state of PDE4. Rats were unilaterally injected in striatum with either a PKA activator (db-cAMP, upper panels) or a PKA inhibitor (Rp-cAMPS, lower panels). PKA-mediated phosphorylation of PDE4 increased both enzyme activity and the affinity of ^{11}C-(*R*)-rolipram binding about 10-fold. Both the PKA activator and inhibitor had the expected effect on radioligand uptake in striatum injected with drug compared to the side injected with saline. These effects were not merely caused by altered blood flow, given that they were not found using the inactive enantiomer ^{11}C-(*S*)-rolipram (right panels).

In Vivo Phosphorylation Status. We further examined the effects of PKA modulators in conscious rats on ^{11}C-(*R*)-rolipram binding compared to the much less active enantiomer ^{11}C-(*S*)-rolipram (Fig 2) [12]. Two drugs were studied. db-cAMP, which is a cAMP analogue, was used to activate PDE4, thus increasing activity of the cAMP-dependent PKA. PKA then phosphorylated the PDE4 enzyme and increased PDE4 activity. We also studied Rp-cAMP, a PKA inhibitor that directly inhibits PKA function, thereby decreasing PDE4 activity. Unilateral injection of the PKA activator (db-cAMP) and the PKA inhibitor (Rp-cAMP) into the striatum significantly increased and decreased ^{11}C-(*R*)-rolipram binding, respectively. These effects were not caused by changes in blood flow or delivery of radioligand to the brain, because these agents did not affect ^{11}C-(*S*)-rolipram binding. These results supported the importance of measuring *in vivo* ^{11}C-(*R*)-rolipram binding in the brain to assess response to physiological or pharmacological challenges to the cAMP second messenger system.

PDE4 Binding in MDD. Based on these preclinical findings, we hypothesized that ^{11}C-(*R*)-rolipram PET in humans would provide a unique *in vivo* measure of PDE4 density and affinity not possible in postmortem tissue. Expanding our work, we sought to quantify the binding of ^{11}C-(*R*)-rolipram as an indirect measure of this enzyme's activity in the brain of individuals with MDD compared with healthy controls [13]. This is particularly important because animal studies had suggested that upregulation of the cAMP cascade, including PDE4, was a mechanism of action common to several antidepressant treatments. To avoid the misleading results that can be obtained from small sample sizes, we have now scanned a total of 44 unmedicated, moderately depressed patients with MDD and 35 age- and gender-matched healthy controls, which is about twice the size of most PET studies in psychiatry. Notably, about half the patients were treatment-naïve. ^{11}C-(*R*)-rolipram binding in the brain was measured using arterial ^{11}C-(*R*)-rolipram levels to correct for the influence of cerebral blood flow. MDD subjects showed a widespread, 18% reduction in ^{11}C-(*R*)-rolipram binding (p=0.001) that was not caused by different gray matter volumes (Fig 3). Decreased rolipram binding of similar magnitude was observed in most brain areas. Rolipram binding did not correlate with the severity of depressive or anxiety symptoms. These results were the first to demonstrate that brain levels of PDE4, a critical enzyme that regulates cAMP, are decreased in unmedicated individuals with MDD *in vivo*. Furthermore, the results are in line with human postmortem and rodent studies demonstrating downregulation of the cAMP cascade in MDD, and support the hypothesis that PDE4 inhibitors—which increase cAMP cascade activity—may have antidepressant effects.

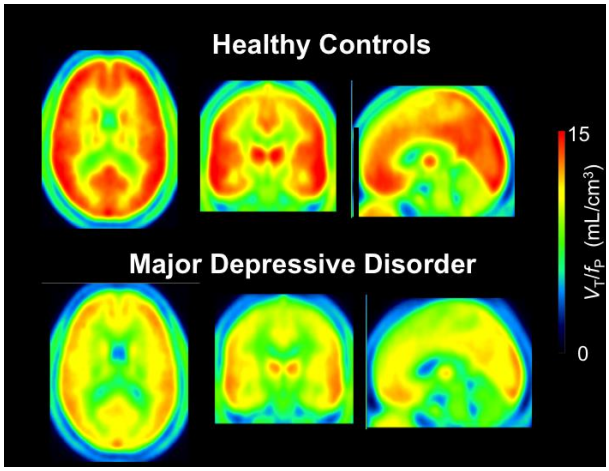

Fig 3. ^{11}C-(*R*)-rolipram binding was globally decreased by 18% in brains of unmedicated MDD patients during a major depressive episode (n = 44) compared to matched controls (n = 35; p = 0.001). These mean parametric images of the two groups represent the "absolute" quantitation of radioligand binding calculated on an individual voxel level and using the concentration of radioligand in serial arterial blood samples.

The Effect of Chronic Antidepressants on PDE4 Binding Building on this work, we sought to determine if antidepressant treatment upregulates PDE4 in humans as it does in animals. In addition to the rolipram PET scans without medication reported above, 23 of the 44 unmedicated MDD patients had a follow up rolipram scan after starting treatment with SSRIs. Preliminary analyses show that these patients had a 12 ± 36% increase in rolipram binding after SSRI treatment across all brain regions ($p < 0.001$ when age was used as a covariate). The change in rolipram binding after SSRI varied markedly among patients, as indicated by the large standard deviation of 36%. In contrast, 13 healthy controls who had a repeat scan without SSRI showed similar binding on repeat scans with changes of only -1 ± 13%. Age affected the magnitude of SSRI-induced increase in rolipram binding; older patients showed greater increases after SSRI ($p \leq 0.001$). However, no correlation was observed between rolipram binding and symptom severity, neither for the unmedicated baseline scan nor in response to SSRI treatment, possibly because of the heterogeneity of the disorder or the heterogeneity of PDE4, which has four distinct subtypes.

Taken together, these results elucidate two important and related points. First, the cAMP cascade, as indirectly measured with PDE4 binding, was downregulated in unmedicated patients with MDD. Second, antidepressant treatment normalized this downregulation. These studies suggest that PDE4 inhibition, perhaps via subtype selective agents, might again be assessed for efficacy in MDD; the results also broadly support the cAMP theory of depression and of antidepressant action. Additionally, cognitive benefits may be elicited over a broad range of disorders including Alzheimer's disease, schizophrenia, and Parkinson's disease.

Development of a novel PET ligand to selectively image PDE4D

MIB/NIMH and Tetra Discovery Partners developed several PDE4D selective PET ligands and tested them in monkey PET scans. Among these candidates, ^{11}C-T-1650 showed the best results. Because PDE4 type B and type D have similar structures, type D selective ligands were developed based on 3D structural differences between type D and B [3]. Based on in vitro experiments using recombinant DNAs of human PDE4B and D, T-1650 has 300 times greater affinity to PDE4D than to PDE4B. Monkey PET scans showed moderate brain uptake peaking at 3 SUV and was washed out to 1/3 of the peak in two hours, which allowed precise quantification of ligand binding, including both association to and dissociation from PDE4D (Figs. 4 and 5). Both V_T (total uptake) and BP_{ND} (ratio of specific to nonspecific uptake) of ^{11}C-T-1650 were greater than that of ^{11}C-(*R*)-rolipram, even though T-1650 binds to only PDE4D, while rolipram binds to all PDE4 subtypes (Table 1). As described above, ^{11}C-(*R*)-rolipram has been successfully used in rodent, healthy humans, and patients. Therefore, ^{11}C-T-1650 is expected to be useful to study PDE4D in human.

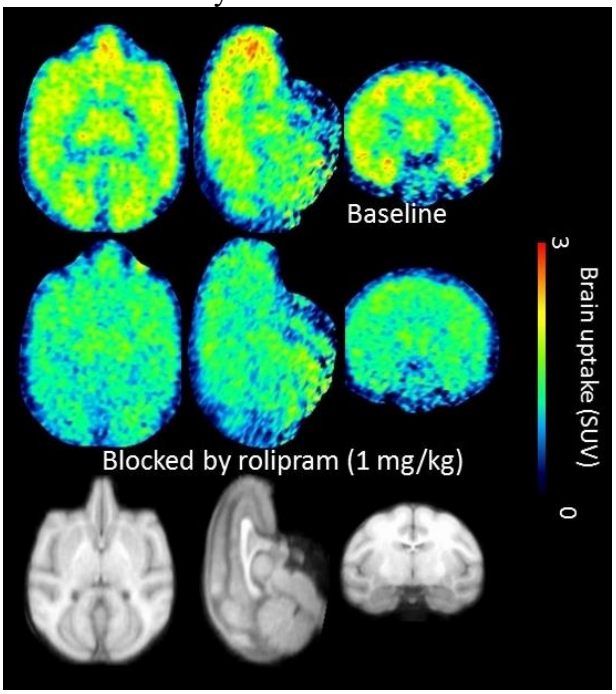

Fig. 4. Specific binding of ^{11}C-T1650 was blocked with rolipram (1 mg/kg).

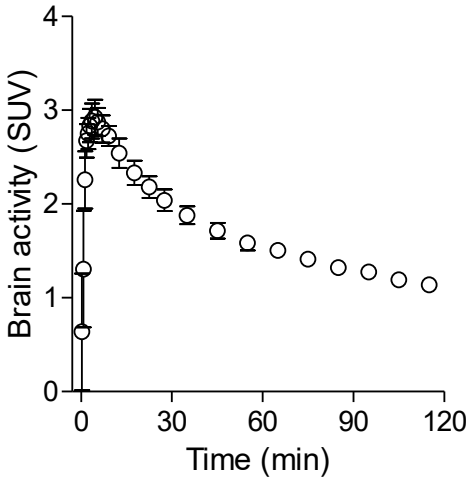

Fig. 5. ^{11}C-T-1650 showed good brain uptake and clear washout in all brain areas.

As background, we previously studied a radioligand for another subtype of PDE4 (i.e., PDE4B) in protocol 17-M-0041, which was terminated. The primary outcome measures were identifiability and time stability of distribution volume (V_T). This radioligand for PDE4B had

significant flaws in healthy human subjects, based on studies here and at the Karolinska Institutet. Specifically, time stability of V_T was consistent with radiometabolites accumulating in the brain. In addition, Pfizer will not allow us to use their therapeutic candidate, which also has major limitations (low bioavailability and short half-life). For these two reasons, we terminated the protocol using the PDE4B radioligand.

Table 1. Total distribution volume (V_T) and specific-to-nondisplaceable ratio (BP_{ND}) of ^{11}C-T-1650 and ^{11}C-(*R*)-rolipram in monkey

	V_T		BP_{ND}	
	T-1650	(*R*)-Rolipram	T-1650	(*R*)-Rolipram
Prefrontal cortex	10.8	4.9	1.6	0.6
Temporal cortex	9.7	5.1	1.3	0.7
Putamen	6.7	5.5	0.6	0.8

V_{ND} measured by Lassen occupancy plot by blocking with rolipram (1 mg/kg):
^{11}C-T-1650: 4.2; ^{11}C-(*R*)-rolipram: 3.0

Development of PDE4D inhibitor BPN14770 and initial tests in animals

BPN14770 is a first-in-class, subtype selective, phosphodiesterase type-4D-negative allosteric modulator (PDE4D-NAM)[14]. The unique mechanism of action and subtype selectivity distinguishes BPN14770 from the two approved PDE4 inhibitors, roflumilast (Daliresp™) and apremilast (Otezla™). Roflumilast and apremilast are competitive inhibitors that inhibit the PDE4 enzymes by binding in the active site competitively with cAMP. As the amino acid sequence of the PDE4 active site is conserved between the four subtypes, roflumilast and apremilast inhibit all subtypes of PDE4 equally strongly. Both compounds cause emesis in humans and other species, and as a class, PDE4 competitive inhibitors have been found to cause mesenteric vasculopathy in rats, dogs and primates, although this toxicity has not been observed in humans. In contrast, BPN14770 does not cause emesis in ferrets, cynomolgous monkeys or marmosets and does not cause mesenteric vasculopathy in rats or dogs in toxicological studies of 28 days duration. Thus, BPN14770 presents a unique procognitive and preclinical safety profile.

Clearance of BPN14770 by hepatocytes from human, rat, dog and monkey, expressed as hepatic extraction ratio (EH), was 16%, 8.2%, 17%, and 12%, respectively. Qualitative metabolite profiling in human, rat, dog, and monkey hepatocytes showed a single metabolite in human (<1% parent). BPN14770 was highly bound to plasma proteins in all species tested. The fraction unbound (free) was 0.5, 0.4, 0.2, and 0.5%, for mouse, rat, dog, and human, respectively.

BPN14770 is highly bioavailable in rats, mice and dogs (F% = 100), distributes to the brain (B/P = 0.32-0.48), and has a plasma $t_{½}$ of 4.8 hours in rats, 10.9 hours in mice, and 11 hours in dogs. The multi-species pharmacokinetic data suggest that BPN14770 will have adequate half-life for once daily oral administration in humans. In bile duct cannulated rats, BPN14770 was found to be eliminated as a conjugate through the bile. Only the unchanged drug was detected in plasma, and no BPN14770 was detected in urine.

BPN14770 has cognitive benefit in the mouse Novel Object Recognition (NOR) test at 0.3 mg/kg PO and above as shown by an increase in the Discrimination Index. BPN14770 C_{max} at the minimum effective dose (MED) was 160 ng/mL in plasma and 40 ng/g in the brain. Cognitive benefit is maintained after chronic dosing for 7 days. BPN14770 also has benefit in a mouse model of cholinergic impairment, the scopolamine-impaired Y-maze (male C57Bl6 mice MED = 1 mg/kg).

BPN14770 did not affect cardiovascular function in beagle dogs at doses up to 100 mg/kg, nor did the compound affect respiratory function or exert neuropharmacologic effects in rats at doses up to 60 mg/kg. In the in vitro hERG assay, an increase in current of 12% and 33% was seen at concentrations of 10 and 30 μM, respectively. No IC_{50} could be calculated due to this increase in current.

In a rat 28 day toxicology study BPN14770 was well tolerated up to 60 mg/kg with no deaths on study, and no hematology, clinical chemistry, liver weight or gross necropsy findings. There were no microscopic findings. Unlike competitive PDE4 inhibitors such as roflumilast (Daxas™) and apremilast (Otezla™), BPN14770 did not cause mesenteric vasculopathy at the doses studied.

In a dog 28 day toxicology study, the no observed adverse effect level (NOAEL) for BPN14770 was 30 mg/kg. At 100 mg/kg BPN14770 caused inappetence and weight loss and in female dogs an approximately 30 msec increase in QTc with a concomitant increase in heart rate of 10 bpm. Based on the lack of inhibition of hERG and the associated gastrointestinal disturbance, this change likely reflects an indirect effect on QTc. There were no changes in hematology and ophthalmology. Macroscopic observations included pale livers in two of eight animals at 100 mg/kg which correlated with mild alanine transaminase (ALT) elevation only in those two animals. Microscopic changes of peri-portal hepatocellular cytoplasmic vacuolation were observed in the livers of one male and two female dogs at 100 mg/kg. No liver changes were observed in recovery dogs suggesting that these changes, if present, were reversible.

Clinical trials of BPN14770 in healthy subjects

Tetra Discovery has completed the following two trials of BPN14770 in healthy subjects.

BPN14770-CNS-101: a randomized, double-blind, placebo controlled, ascending single dose study to evaluate the safety, tolerability, and pharmacokinetic profile of BPN14770. in healthy subjects (18 – 55 years old).

BPN14770-CNS-102: a randomized, double-blind, placebo-controlled, multiple ascending dose study to evaluate the safety, tolerability, and pharmacokinetic profile of BPN14770 in healthy young (25 – 44 years old) and elderly (60 – 78 years old) male and female subjects, and to provide a preliminary assessment of the cognitive effects of BPN14770 in healthy elderly subjects. Each subject was randomized to receive a fixed daily dose of either BPN14770 or placebo twice daily (every 12 hours, or BID) or once daily (every 24 hours, or QD) for 8 consecutive days

The following is the summaries of the results of these studies reported in the Investigator's Brochure.

BPN14770-CNS-101:

Twenty-four subjects received BPN14770 (six at each dose level: 5mg/15mg/50mg/100mg) and eight subjects received placebo. Detectable plasma BPN14770 concentrations were measured after dosing starting between 0.5 hour and 1 hour after dosing, continuing through 48 hours after dosing for all four dose levels. Drug absorption was variable but moderately rapid with median T_{max} values ranging from 1.5 to 3 hours. The plasma pharmacokinetics of BPN14770 after oral administration appeared to be linear for single doses ranging from 5 to 100mg, although a slight trend for a greater than proportional increase in exposure was observed in the 100mg group as compared to the lower dose groups. The apparent terminal elimination half-life of BPN14770 was consistent among dose groups and averaged between 11 and 13 hours.

The following adverse events were reported.

Table 2. Adverse events after one oral dose of BPN14770

Category	5 mg N=6	15 mg N=6	50 mg N=6	100 mg N=6	Total (active drug) N=24	Placebo N=8
Number (%) of subjects with:						
At least one AE	2 (33.3)	1 (16.7)	2 (33.3)	4 (66.7)	9 (37.5)	3 (37.5)
At least one drug-related AE[a]	1 (16.7)	1 (16.7)	1 (16.75)	4 (66.7)	7 (29.2)	2 (25.0)
At least one severe AE	0	1 (16.7)	0	0	1 (4.2)	0
At least one AE leading to subject discontinuation	0	0	0	0	0	0
Number of adverse events:						
Total number of AEs	3	1	3	8	15	6
Total number of drug-related AEs[a]	1	1	2	8	12	2
Total number of serious AEs	0	0	0	0	0	0

AE = adverse event, N = Number of Subjects.
Adverse event mapping was based on the Medical Dictionary for Regulatory Activities (MedDRA) version 18.1 thesaurus.
[a] Drug-related AEs were reported by the Investigator as possibly, probably, or definitely related to treatment.

AEs that occurred in more than one subject that were considered related to treatment consisted of nausea (reported by 4 of 6 subjects in the 100mg dose group), vomiting (reported by 2 of 6 subjects in the 100mg dose group, both of which also reported nausea) and diarrhea (reported by 2 of 8 subjects in the placebo group and 1 subject each in the 5mg and 50mg BPN14770 dose groups). All but one treatment-emergent AE were mild or moderate in severity. One severe AE was reported for one subject in the 15mg dose group, who reported intermittent severe renal colic starting approximately 24 hours after dosing.

BPN14770-CNS-102:

Table 3. Overview of study cohorts for 8-day oral administration of BPN14770

Cohorts/Dose Levels			Number of Subjects		
Cohort	**Description**	**BPN14770 Dose**	**BPN14770**	**Placebo**	**Total**
1	Young	15 mg BID	6	2	8
2	Young	30 mg BID	6	1[1]	7[1]
3	Young	50 mg BID	6	2	8
4a[2]	Elderly	10 mg BID	6	1	7
4b[2]	Elderly	10 mg BID	4	4	8
5a	Elderly	20 mg BID	6	2	8
5b	Elderly	20 mg BID	4	3	7
6a	Elderly	40 mg BID	6	2	8
6b	Elderly	40 mg BID	4	3	7
7 (optional)[3]	Young	75 mg QD	6	2	8
Total (maximum)			54	23	77

BID = twice daily (every 12 hours); QD = once daily (every 24 hours).

[1] Violation of eligibility criteria for one subject on Day 1 led to dosing only 7 subjects (6BPN14770, 1 placebo) in Cohort 2.

[2] Cohort 4a included 7 of the planned 8 subjects (6 active-treated and 1 placebo-treated); therefore, Cohort 4b was increased by one subject, so that a total of 10 subjects received active drug and 5 subjects received placebo at the 10 mg BID dose level.

[3]An additional cohort of young subjects was enrolled after completion of Cohorts 1-6.

The following results were obtained:

1. Multiple doses of BPN14770, were administered twice daily (15 mg, 30 mg, or 50 mg) or once daily (75 mg) to healthy young subjects and twice daily (10 mg, 20 mg, or 40 mg) to healthy elderly subjects.

2. There was evidence of greater than proportional increases in BPN14770 exposure after a single dose or repeated dosing. Drug accumulation was approximately 2-fold after QD dosing. After BID dosing, moderate drug accumulation (approximately 3- to 5-fold) was observed. Steady state appeared to be reached within 2 to 4 days of BID or QD dosing. When adjusted for differences in dose, mean BPN14770 exposure appeared to be similar between healthy young and healthy elderly subjects.

The following adverse events were reported:

Table 4. Adverse events in 8-day oral administration of BPN14770

Category	Young Subjects					Elderly Subjects			
	15 mg BID N=6	30 mg BID N=6	50 mg BID N=6	75 mg QD N=6	Placebo N=7	10 mg BID N=10	20 mg BID N=10	40 mg BID N=10	Placebo N=15
Number (%) of subjects with:									
At least one AEs	2 (33.3)	4 (66.7)	2 (33.3)	4 (66.7)	0	5 (50.0)	3 (30.0)	9 (90.0)	6 (40.0)
At least one drug-related AE[a]	2 (33.3)	1 (16.7)	2 (33.3)	4 (66.7)	0	4 (40.0)	2 (20.0)	7 (70.0)	4 (26.7)
At least one mild AE	2 (33.3)	4 (66.7)	2 (33.3)	3 (50.0)	0	4 (40.0)	3 (30.0)	8 (80.0)	6 (40.0)
At least one moderate AE	0	0	0	1 (16.7)	0	1 (10.0)	0	1 (10.0)	0
At least one serious or severe AE	0	0	0	0	0	0	0	0	0
At least one AE leading to subject discontinuation	0	0	0	0	0	0	0	0	0
Deaths	0	0	0	0	0	0	0	0	0
Number of adverse events:									
Total number of AEs	3	8	2	14	0	13	4	16	8
Total number of drug-related AEs[a]	3	3	2	12	0	8	2	11	6
Total number of mild AEs	3	6	2	10	0	11	4	15	8
Total number of moderate AEs	0	0	0	2	0	2	0	1	0
Total number of serious AEs	0	0	0	0	0	0	0	0	0

AE = adverse event; BID = twice daily; N = Number of Subjects; QD = once daily.
Adverse event mapping was based on the Medical Dictionary for Regulatory Activities (MedDRA) version 19.0 thesaurus.
[a] Drug-related AEs were reported by the Investigator as possibly, probably, or definitely related to treatment.

No deaths or other serious adverse events were reported across the young and elderly cohorts, and no subject withdrew from the study due to adverse events. The majority of AEs were of mild severity. Two moderate AEs (nausea and vomiting) were reported by 1 subject in the 75-mg QD dose group; no other AEs of moderate severity were reported across the young cohorts.

Of the 27 adverse events reported by young subjects who received active drug, a total of 20 AEs observed in 9 subjects were considered by the Investigator as related to study treatment.
No clinically significant effects of multiple oral doses of BPN14770 administered twice daily (15 mg, 30 mg, or 50 mg) or once daily (75 mg) to healthy young subjects and twice daily (10 mg, 20 mg, or 40 mg) to healthy elderly subjects were observed on biochemistry, hematology, or urinalysis; vital signs; or ECGs.

Overall, no deaths or serious adverse events were reported during the study. No subjects withdrew from the study due to adverse events, and all treatment-emergent adverse events resolved without sequelae by the follow-up visit. One subject withdrew consent for treatment, which the Investigator determined was not a withdrawal for an AE, although she had a headache which had not fully resolved.

Adverse events that occurred in more than 1 subject within a dose group or placebo group that were considered related to study drug were limited to the 40-mg BID dose group. Headache was reported in 4 of 10 subjects; diarrhea, increased alanine aminotransferase and aspartate aminotransferase, and hot flush were each reported in 2 of 10 subjects.

The majority of AEs were of mild severity. A total of 3 moderate AEs were reported across the elderly cohorts; 2 moderate AEs (hypotension, dizziness) were reported by 1 subject in the 10-mg BID dose group, and 1 moderate AE (headache) was reported by 1 subject in the 40-mg BID dose group.

The rationale for selecting the dose of BPN14770 in this PET protocol

We will administer 50 mg BID of BPN14770 for the following reasons. In a multiple dose study in healthy volunteers, a dose of 50 mg bid for 8 days was very well tolerated in young individuals (25 to 44 years old). Only 2 of 6 reported any AE: one mild diarrhea and one mild nausea. Both AEs were transient and neither led to discontinuation. In elderly individuals (age >60) BPN14770 40 mg was also well tolerated over 8 days, with no discontinuations for adverse events. The most common AE was transient headache in 4 of 10 individuals; 2 of 10 reported diarrhea compared to 1 on placebo.

2. Study Objectives

a. Primary objectives

This study has three primary objectives. First, we will determine whether the uptake of ^{11}C-T-1650 in the brain reflects the distribution of PDE4D, as demonstrated by blocking with a PDE4D selective medication BPN14770. Second, we will measure binding site occupancy of BPN14770 and compare these data to plasma levels of the drug. Third, we will measure the test/retest reproducibility of brain uptake quantified by kinetic modeling and using arterial blood samples.

b. Secondary objectives

The secondary objective is to estimate dosimetry (i.e., radiation-absorbed doses) of ^{11}C-T-1650.

3. Subjects

a. Description of study populations

We propose to study four groups of participants:

Phase 1: One subject will have a whole body PET scan with 10 mCi of ^{11}C-T-1650 to confirm that no organ has prominently high uptake of ^{11}C-T-1650.

Phase 2: 15 healthy subjects will have three brain PET scans, baseline, about 90-180 min after the first dose of BPN14770 (Scan #2), and after three and a half-day administration of BPN14770 (Scan #3) to measure specific binding and BP_{ND} of ^{11}C-T-1650 and binding site occupancy of BPN14770. The dose of BPN14770 is 50 mg BID (except for the last day in which subjects will take a single dose). BPN14770 levels will be measured in plasma samples.

Phase 3: Nine healthy subjects will have a whole body scan using 20 mCi ^{11}C-T-1650 to estimate radiation-absorbed doses.

Phase 4: 15 healthy subjects will have two brain PET scans; test-retest kinetic brain imaging with arterial blood sampling.

Note: As described below in Section 10 (“Statistical Analysis”), these requested numbers account for and include subjects who may drop out.

b. Inclusion criteria

<u>All phases</u>

1. Age ≥ 18.
2. Able to give written informed consent.
3. Medically and psychiatrically healthy.

4. Enrolled in 01-M-0254 "The Evaluation of Participants with Mood and Anxiety Disorders and Healthy Volunteers" (PI: Dr. Carlos Zarate) or 17-M-0181 "Recruitment and Characterization of Healthy Research Volunteers for NIMH Intramural Studies" (PI: Dr. Joyce Chung).

Additional inclusion criterial for Phase 2

1. Age ≤ 55.
2. Body mass index between 18 kg/m^2 to 32 kg/m^2, inclusive, and body weight of ≥50 kg (110 pounds).
3. Female subjects must be surgically sterile (bilateral tubal ligation, hysterectomy, or bilateral oophorectomy at least 6 months prior to dosing of BPN14770), at least two years post-menopausal, or willing to use two barrier methods of contraception from initial screening until one month after taking the last dose of study drug.
4. Male subjects must be willing to inform female partners of their participation in the study and must agree to use adequate contraceptive methods (vasectomy performed at least 6 months prior to dosing BPN14770 or use at least one barrier method of birth control).

c. Exclusion criteria –All phases

All phases

1. Clinically significant laboratory abnormalities based on the following tests (performed under screening protocol 01-M-0254 or 17-M-0181): CBC; acute care panel; hepatic panel; mineral panel; urinalysis; urine drug screen; urine pregnancy test (females); and lipid panel; hepatitis panel (A, B, C); syphilis screening test; total protein; uric acid; creatine kinase; cholesterol; thyroid panel; prothrombin and partial prothrombin tests; and EKG.
2. Have a brain disease (such as multiple sclerosis or stroke).
3. Any current Axis I diagnosis, based on interview and self-reporting performed under screening protocol 01-M-0254 or 17-M-0181.
4. Positive HIV test.
5. Current or past history of significant cardiovascular, cerebrovascular, pulmonary, renal, or liver disease. Stable, well-controlled hypertension and hyperlipidemias are allowed.
6. Taking psychotropic drugs (i.e. benzodiazepines or antidepressants) including sedative antihistamines; moderate to strong inhibitors or inducers (i.e. fluconazole or ciprofloxacin) of any CYP450 enzyme. A complete listing of such inhibitors or inducers may be found in Attachment 1, List of P450 inhibitors.
7. Recent exposure to radiation related to research (e.g., PET from other research) that, when combined with this study, would be above the allowable limits.
8. Inability to lie flat on camera bed for at least two hours.
9. Pregnancy or breastfeeding.
10. Positive screen for drugs of abuse or cotinine (at screen or upon admission), or a positive alcohol result (upon admission).
11. Current use of psychiatric medications.
12. NIMH employees and staff or immediate family members of NIMH employee/staff.

Additional exclusion criterial for Phase 2 and 4

1. coagulation disorder;

2. thrombocytopenia;
3. Found to have inadequate collateral circulation of the radial artery (see section 5.c screening below)
4. Are unable to have an MRI scan (e.g., pacemakers or other implanted electrical devices, brain stimulators, dental implants, aneurysm clips (metal clips on the wall of a large artery), metallic prostheses (including metal pins and rods, heart valves, and cochlear implants), permanent eyeliner, implanted delivery pumps, or shrapnel fragments, metal fragments in the eye)

Additional exclusion criterial for Phase 2

1. Marked bradycardia (heart rate <45 beats per minute [bpm]) or tachycardia (heart rate >110 bpm) based on supine ECG values obtained at Screening, before the first dose of BPN14770 on the day of the study. Out-of-range vital signs may be repeated once at each eligibility assessment (prior to the start of dosing).
2. History of hematological disorders (e.g., thrombocytopenia) in the immediate family (i.e., parents and siblings).
3. Clinically important or significant conduction abnormalities on single ECG (including QTc interval >450 msec) or evidence or history of long QT syndrome. This exclusion applies to the ECGs obtained at Screening, before the first dose of BPN14770 on the day of the study.
4. Current or past history of gastric or duodenal ulcers or other diseases of the gastrointestinal tract that could interfere with absorption of study drug. Note: Subjects with a history of appendectomy or cholecystectomy may be enrolled.
5. Active acute or chronic infectious diseases.
6. Any history of alcohol or drug abuse within the previous year prior to the Screening visit (per the current edition of the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition: DSM-5), or regular (daily) consumption of alcohol exceeding two bottles of beer, or the equivalent amount of other forms of alcohol (1 serving = 12 oz beer, 5.0 oz wine, or 1.5 oz distilled spirits).
7. Unwilling to abstain from alcohol within 72 hours of before the first dose of BPN14770.
8. Currently ingest nicotine in any way (including smoking cigarettes, vaping, and via patch), or have ingested any nicotine products within the past 3 months.
9. Participation in other clinical studies involving investigational drug within the previous 30 days prior to the first day of the study.
10. Unwilling to forgo donation of blood or blood products (including plasma) during the 8 weeks before the first day of the study.
11. History of allergy to penicillin or sulfonamides, or any other clinically significant drug allergy that includes symptoms such as shortness of breath, rash, or edema.
12. A suicidal ideation intensity score of 2 or higher per screening C-SSRS assessment and/or any suicidal behavior within the past 30 days.

4. Study Design and Methods

a. Study overview

This is a single center study in healthy volunteers. Healthy volunteers will be screened under protocol 01-M-0254 or 17-M-0181. As noted above, participants must meet inclusion and exclusion criteria and sign an informed consent document. The four phases of this study will be:

1) initial first-in-human whole-body imaging of one subject with a low injection activity of 10 mCi;

2) brain imaging at baseline (Scan #1), about 90-180 min after the first dose of BPN14770 (Scan #2), and after three and a half-day administration of BPN14770 (Scan #3) to measure specific binding and BP_{ND} of ^{11}C-T-1650 and binding site occupancy of BPN14770. Binding site occupancy in the brain will be compared to plasma levels of BPN14770. The measurement of BP_{ND} by Lassen occupancy plot [15] assumes no change in PDE4D density after administering BPN14770. Comparison between Scan #1 (baseline) and Scan #2 (90-180 min after the first dose of BPN14770) allows the measurement of BP_{ND} with little changes in PDE4D density. Comparison between Scan #1 and Scan #3 allows measurement of binding site occupancy by BPN14770 at a steady state level.

3) whole body imaging to estimate radiation-absorbed doses of ^{11}C-T-1650, and

4) test-retest kinetic brain imaging with arterial blood sampling.

In the past, the NIH Radiation Safety Committee (RSC) required that whole body imaging be performed first in several subjects for any new radioligand to estimate radiation exposure to the body. However, if the radioligand subsequently proved ineffective for brain imaging, then these subjects who underwent whole body imaging would have been unnecessarily exposed to radiation. For this reason, the RSC allowed brain imaging using new ^{11}C- and ^{11}F-labeled radioligands to be performed after doing whole body imaging in one subject in order to determine whether the radioligand was worth pursuing as a brain imaging agent. That is, 'go / no-go' decisions are made on a small number of subjects based on brain imaging. The request to do so in this protocol follows the identical steps approved for another protocol (14-M-0068, "Evaluation of a Novel Positron Emission Tomography (PET) Radiotracer for TARP γ-8").

We propose to study up to 40 healthy volunteers: one in Phase 1 (whole body imaging with a low injection activity), 15 in Phase 2 (baseline and blocked brain scans), 9 (whole body imaging to estimate radiation-absorbed doses, and 15 in Phase 4 (test-retest kinetic brain imaging with arterial blood sampling).

To estimate the radiation exposure from this new radioligand, we will follow the prior suggestions from our laboratory [16], which have been approved by the NIH RSC. Specifically, we found that the average exposure of all published human studies with ^{11}C-radioligands more accurately reflected the exposure to humans than estimation based on imaging in monkeys with that particular radioligand.

Phase 1: One subject will have a first-in-human whole body PET scan with 10 mCi ^{11}C-T-1650 to confirm that no organ has prominently high uptake of ^{11}C-T-1650.

Phase 2: 15 healthy subjects will have three brain PET scans, baseline (Scan #1), about 90-180 min after the first dose of BPN14770 (Scan #2), and after three and a half-day administration of BPN14770 (Scan #3). In each scan, 20 mCi of ^{11}C-T-1650 will be injected. In Scan #1 and #2, the binding will be measured by kinetic brain imaging with arterial blood sampling. In addition, in all of Scan #1, 2, and 3, by using venous samples, radioligand binding will be measured as brain uptake (SUV) normalized to venous concentrations of ^{11}C-T-1650 because an arterial line will not be placed in Scan #3 due to three-day interval between Scan #2 and 3. Venous samples will be analyzed for BPN14770 levels. The dose of BPN14770 will be 50 mg BID (except for the last day in which subjects will take a single dose).

Phase 3: Nine healthy subjects will have a whole-body scan using 20 mCi of ^{11}C-T-1650 to estimate radiation-absorbed doses.

Phase 4: 15 healthy subjects will have two brain PET scans; test-retest kinetic brain imaging with arterial blood sampling.

If Phase 2 shows substantial specific (i.e., displaceable) binding, we will be confident that the radioligand itself can be used to measure the density of PDE4D in human subjects, including patients with MDD. For such potential studies, we will need to determine dosimetry and test / retest variability of the PET scans, independent of the results from three-day administration of BPN14770. Thus, if Phase 2 shows substantial (>40%) specific binding in three subjects, we will simultaneously proceed with Phases 2, 3, and 4.

Brain MRI and PET scans are part of the protocol. There will be no brain MRI in the whole body dosimetry studies in Phases 1 and 3. The MRI will be obtained within 12 months of the PET scans (i.e., up to 12 months before or after the PET scan) and can be performed on the same day. Brain PET scans will be performed with vital sign monitoring. In Phase 2 and 4, ^{11}C-T-1650 brain PET scans will be associated with arterial sampling for the input function measurement except Scan #3 in Phase 2. No arterial input function will be taken for the Phases 1 or 3 studies, however, several venous samples may be obtained in these studies to guide the analysis of arterial samples obtained in the brain scans.

Number of visits and time commitment of participants.
The visits are described below and summarized in Table 5.
Phase 1: whole body imaging with 10 mCi injection
1st outpatient visit: pregnancy test; whole body PET (4 hours)

Phase 2: brain imaging (20 mCi) at baseline and after BPN14770
1st outpatient visit: pregnancy test; MRI (2 hours)
2nd outpatient visit: pregnancy test; baseline brain PET imaging (4 hours)
3rd outpatient visit: pregnancy test, brain PET imaging starting approximately 90-180 min after the first dose of PDE4D selective inhibitor BPN14770 (6 hours). BPN14770 (50 mg BID) administration will be continued for three and a half days (the last day subjects will take a single dose).
4th outpatient visit: On the last day of BPN administration. The third PET scan will be started about 90-180 min after the last dose of BPB14770 (4 hours)

5th outpatient visit: 3 -7 days after the last dose of BPN14770

The first two brain PET scans may be performed on a single or two different days, based on the availability of the subject and the PET camera. The order of the visits for MRI and PET scans may be changed based on the availability of the scanners. The baseline PET scan and the first PET scan after BPN14770 administration may occur on the same day and, if so, be separated by at least 2.5 hours to allow for decay of the first injection ($T_{1/2}$ = 20 min).

Phase 3: whole body imaging with 10 mCi injection
1st outpatient visit: pregnancy test; whole body PET (4 hours)

Phase 4: test retest kinetic brain imaging (20 mCi)
1st outpatient visit: pregnancy test; MRI (2 hours)
2nd outpatient visit: pregnancy test; brain PET (4 hours)
3rd outpatient visit: pregnancy test; retest brain PET (4 hours)

The PET scans may be performed on one day or on two different days. The order of the visits for MRI and PET scans may be changed based on the availability of the scanners. Both PET scans may occur on the same day and, if so, be separated by at least 2.5 hours to allow for decay of the first injection ($T_{1/2}$ = 20 min).

Table 5. Number of visits and time commitment of participants

	Visit #1	**Visit #2**	**Visit #3**	**Visit #4**	**Visit #5**
Phase 1	Pregnancy test; Whole-body PET scan *(4 hours)*	N/A	N/A	N/A	N/A
Phase2	Pregnancy test; Brain MRI *(2 hours)*	Pregnancy test; Brain PET scan #1 *(4 hours)*	Pregnancy test; BPN14770 admin.; Brain PET scan #2 *(6 hours)*	Pregnancy test; Brain PET scan #3 *(4 hours)*	Examination 3-7 days post last dose of BPN14770
Phase 3	Pregnancy test; Whole-body PET scan *(4 hours)*	N/A	N/A	N/A	N/A
Phase 4	Pregnancy test; Brain MRI *(2 hours)*	Pregnancy test; Brain PET scan #1 *(4 hours)*	Pregnancy test; Brain PET scan #2 *(4 hours)*	N/A	N/A

* The MRI may be scheduled on the same day as a PET scan
** MRI does not need to be performed if participant has had an adequate MRI within 12 months of the brain PET scan.

b. Recruitment

Healthy volunteers will be recruited under 01-M-0254 or 17-M-0181 and not under the current protocol. NIH Employees/staff will not be directly recruited by or through their supervisors or co-workers to participate in this study.

c. Screening

Healthy volunteers will be screened under protocol 01-M-0254, "The Evaluation of Participants with Mood and Anxiety Disorders and Healthy Volunteers" (PI: Dr. Carlos Zarate)

or 17-M-0181 “Recruitment and Characterization of Healthy Research Volunteers for NIMH Intramural Studies” (PI: Dr. Joyce Chung). Basic screening will include physical exam, medical and psychiatric history, and laboratory tests: CBC; acute care panel; hepatic panel; mineral panel; urinalysis; urine drug screen; urine pregnancy test (females); and lipid panel; hepatitis panel (A, B, C); syphilis screening test; total protein; uric acid; creatine kinase; cholesterol; thyroid panel; prothrombin and partial prothrombin tests; and EKG. The radial artery pulse is checked for the presence of adequate ulnar collateral flow and the absence of any metals or foreign objects in bilateral wrists. Screening results will be reviewed by a clinically credentialed investigator before the subject undergoes any specific study procedures.

d. Study procedures

Consent will be obtained before any study procedures are done. Subjects participating in Phase 2 and 4 will receive three (Phase 2) or two (Phase 4) ^{11}C-T-1650 brain PET scans and a brain MRI scan. During the PET scan, arterial blood samples will be drawn. The MRI scan may be obtained before, after, or between the two PET scans. The MRI will be performed with a 3 Tesla magnet at the NIH.

Subjects participating in Phases 1 and 3 will receive one ^{11}C-T-1650 whole body PET scan. No MRI will take place in these dosimetry/enzyme occupancy studies. All procedures are solely for research purposes.

MRI

For participants to Phase 2 and 4, A brain MRI will be obtained for anatomic localization and will be performed on a 3 Tesla scanner located at the NIH Clinical Center and will take about one hour. Participants who have had an adequate brain MRI performed at the NIH within 12 months of the dedicated brain PET scan will be exempt from this part of the protocol and their earlier MRI will be used for anatomical localization. Pregnancy tests: For women able to become pregnant, urine pregnancy testing will be done within the 24 hours prior to any MRI or PET scan. PET and MRI will not be done if the pregnancy test is positive.

PET Procedures

1) Radioligand

^{11}C-T-1650 will be prepared according to the IND and administered via an indwelling intravenous catheter over approximately one minute.

2) PET Brain Imaging

After midnight on the day before, participants are not allowed to take caffeine containing drink because caffeine is a nonselective inhibitor of phosphodiesterases. Dedicated brain imaging will be performed using a PET or PET/CT scanner for two to three hours. Participants will be placed on the scanner bed with their head held firmly in place with a thermoplastic mask fixed to the bed. A ^{68}Ge, ^{137}Cs, or CT transmission scan will be performed to measure and correct for attenuation. Tracer infusions will be performed when the subject is already on the scanner bed. After an intravenous bolus of up to 20 mCi, arterial blood samples will be drawn from the arterial catheter during the PET scan. We will collect about 10-20 arterial samples. The sampling will be performed either at discrete time points and/or continuously. In Phase 2, before and after administration of BPN14770, a few samples (< 7) will be obtained to measure concentration of

BPN14770 during each of second and third PET scans. In addition, during each scan of Phase 2, a few venous samples (< 7) will also be obtained to compare venous data of Scan 3 with those in Scan 1 and 2 because it is difficult to place an arterial line with an interval of three days between Scan 2 and 3. Brain uptake (SUV) normalized to venous blood data will be used to compare scan 1 and 3.

The total amount of sampling volume will be about 150 mL in each brain scan. PET images will be acquired in three-dimensional mode with increased length of frame for a total of approximately two to three hours. If the scan lasts more than two hours, the subject will be offered a break out of the camera for approximately 15 minutes. Vital signs (blood pressure, pulse, and respiratory rate) and EKG (either 3- or 12-lead) will be recorded within three hours before tracer injection, and again about 15, 30, and 120 minutes after tracer injection. After the scan, the arterial and venous lines will be removed and the subject will be instructed to void frequently to minimize radiation exposure.

3) PET Whole Body Imaging

After midnight on the day before, participants are not allowed to take caffeine containing drink because caffeine is a nonselective inhibitor of phosphodiesterases. Whole body scans will be performed with a PET/CT scanner for about two hours. No arterial sampling will be done for dosimetry studies but a few venous samples (< 7) will be obtained to measure the concentration of the radioligand. Both a pre-injection transmission scan and a series of dynamic emission scans will be acquired. Each subject will be imaged in contiguous segments from the top of the head to a point below the gonads. To minimize extraneous motion, subjects will wear a head-holding mask and will have their arms and abdomen wrapped with body-restraining sheets. Almost all subjects find this restraint comforting (i.e., they are not afraid of falling off the table), and none have found it intolerable. Vital signs (blood pressure, pulse, and respiratory rate) and EKG (either 3- or 12-lead) will be recorded within three hours of tracer injection, and again about 15, 30, and 120 minutes after tracer injection.

Pregnancy tests: For women able to become pregnant, urine pregnancy testing will be done within the 24 hours prior to any MRI or PET scan. PET and MRI will not be done if the pregnancy test is positive.

4) Arterial Line Placement

After the presence of adequate ulnar collateral flow has been confirmed, a radial artery catheter will be inserted by the Anesthesiology Department or the Vascular Access Department. Before catheter insertion, the skin at the puncture site will be locally anesthetized.

An arterial line will be placed in all brain scans except Scan 3 in Phase 2. Scan 3 in Phase 2 will be performed without arterial line. Only venous samples will be obtained in Scan 3, and they will be used to compare Scan 3 with Scan 1 and 2.

5) Preparation and administration of BPN14770 (Phase 2)

BPN14770 will be provided by Tetra Discovery to NIH Clinical Center Pharmacy as capsules. Tetra Discovery will provide Quality Assurance to NIH Clinical Center Pharmacy.

Participants will be encouraged to have a light meal prior to enzyme occupancy PET studies with blockade by BPN14770. Participants will be monitored by study clinicians after drug administration and during the PET scans to assess drug associated adverse events. On each

day while subjects take BPN14770 as an outpatient, a phone call will be made to participants who take part in Phase 2 to monitor any possible drug-associated adverse events. Contact information of a study clinician will be provided to participants in case they experience adverse reactions.

If a technical problem causes a scan cancellation (e.g., radiochemistry production failure, PET camera malfunction, weather conditions, or unforeseen event in subject's schedule) after the subject takes BPN14770 for three and a half days, subjects may continue to take BPN14770 for three more days (i.e., BPN14770 administration for six and a half consecutive days) and have Scan #3, if willing.

6) Dietary Guidelines (Phase 2)

• Subjects must not consume beverages or foods containing alcohol or grapefruit from 48 hours prior to the first dose of BPN14770 and within 48 hours after the last dose.
• Morning doses will be taken after completing an overnight fast of at least 8 hours. Evening doses will be administered 12 hours after the morning dose and at least 1 hour after completing the evening meal. During the fasting periods, water will be allowed ad libitum.

7) Lifestyle Guidelines (Phase 2)

• Subjects are to refrain from physical activity greater than their normal level of activity from 48 hours prior to the first dose of BPN14770 until the follow up visit 3 – 7 days after the last dose.

8) Laboratory tests after BPN14770 administration (Phase 2)

• On the day of the first dose of BPN14770, before the dose as well as after the PET scan, the following lab tests will be conducted: CBC; acute care panel; hepatic panel; mineral panel; urinalysis; lipid panel; total protein; uric acid; creatine kinase; cholesterol; thyroid panel.

9) Follow up visit after BPN14770 (Phase 2)

Adverse events (AEs), physical exams, vital signs, ECG readings, and clinical laboratory values will be assessed through the follow-up visit 3 -7 days after the last dose of BPN14770. Clinically significant AEs that occur during the study will be followed until resolution.

e. End of participation

Participants remain under the care of their own physicians during and after participation in this protocol. We will notify participants and, with their written permission, their doctors, of any clinically significant results from any procedure done under this protocol.

5. Management of Data and Samples

a. Storage

We will follow NIH guidelines to prevent identification of study participants and other violations of subject confidentiality. Information will be stored using a confidential case number, and no identifiers (name, address, phone number, etc.) will be used that could allow direct linking of database information to individual subjects. Secure e-mail will be used for all electronic communications of subject information between investigators. Blood samples to measure ^{11}C-T-1650 concentrations will be discarded at the end of the study. Blood samples to

measure BPN14770 will be kept in a locked freezer in MIB/NIMH in the NIH Bethesda campus. Demographic and clinical data will be archived on a password-protected server.

All data will be stored on the NIMH server under password-protected accounts accessible only to the principal investigator and directly involved study personnel to preserve subject privacy. All data are regularly backed up, either by the NIMH system administrator or by NIMH CIT personnel.

b. Data and sample sharing plan

No samples will be saved after completion of the study, and no samples will be shared. De-identified data will be shared with Tetra Discovery Partners, but the de-identified data will not be publicly available until publication of the results. However, de-identified data may be shared with collaborating laboratories at the NIH (e.g., Carlos Zarate) as part of planning for a potential clinical trial. When the data are made publicly available, they may be stored outside of the NIH and/or submitted to NIH-designated repositories and databases. Repositories receiving data from this protocol may be open-access or restricted access.

Genomic data will not be obtained under this protocol.

When data are shared, they will be stripped of identifiers and may be coded ("de-identified") or unlinked from an identifying code ("anonymized"). When coded data are shared, the key to the code will not be provided to collaborators, but will remain at the NIH. Data may be shared with investigators and institutions with an FWA or operating under the Declaration of Helsinki (DoH) and reported at the time of continuing review. Sharing with investigators without an FWA or not operating under the DoH will be submitted for prospective IRB approval. Submission to NIH-sponsored or supported databases and repositories will be reported at the time of the Continuing Review. Submission to non-NIH sponsored or supported databases and repositories will be submitted for prospective IRB approval.

Required approvals from the collaborating institution will be obtained and materials will be shipped in accordance with NIH and federal regulations.

6. Additional Considerations

a. Research with investigational drugs or devices

This study involves two INDs, one for ^{11}C-T-1650 and another for BPN14770. The IND for ^{11}C-T-1650 has not yet been submitted to the FDA. The IND for BPN14770, #127,905, is sponsored by Tetra Discovery Partners, Grand Rapids, MI. The drug will be stored according to the Investigator Brochure, which has been uploaded into PTMS.

b. Gene therapy

Not applicable.

7. Risks and Discomforts

a. Medical examination and laboratory testing

Medical examinations, including phlebotomy for blood analysis, are associated with only minimal risk. There is usually some discomfort when the needle is inserted for phlebotomy.

There is minimal medical risk associated with having an EKG. The patient may feel uncomfortable while the electrodes are attached to the chest. The conductive gel sometimes causes some mild irritation.

b. Radiation exposure risks

Radiation exposure in this protocol will be from ^{11}C-T-1650 and the associated transmission scans. To estimate the radiation exposure from this new radioligand, we will follow the suggestion from our laboratory [17] and approved by the NIH RSC and the FDA. Specifically, we found that the average exposure of all published human studies with ^{11}C-radioligands more accurately reflected radiation exposure than estimation based on imaging in monkeys with that particular radioligand.

The average effective dose from 21 radioligands labeled with ^{11}C was 0.019 rem/mCi [16]. Thus, the radiation exposure from a single injection of 20 mCi yields an effective dose of 0.38 rem, not including the transmission scan (described below), which is well below the NIH limit of 5 rem.

With regard to exposure from the transmission scan, the PET Department recently implemented Dr. Innis's suggestion to decrease the current (amperage) and, thereby, the radiation from the CT. We do not need a high resolution (high current) image for attenuation correction; a low resolution, like that from a line source, is perfectly adequate to correct attenuation in the PET emission scan. With the lowered current, the exposure to the lens of the eye is now 0.26 rem, about 1/3 of the previous value.

We wish to maintain flexibility in the choice of PET cameras. Among the various PET cameras, PET/CT has the highest exposure. The effective doses for one head and one whole body transmission scan from a PET/CT are 0.02 and 0.66 rem, respectively. In addition, we allow for up to two transmission scans in case the subject temporarily leaves the camera (e.g., to urinate) or moves significantly.

The radiation exposure from the four phases of this protocol is as follows:

Phase 1: Whole Body Scan. The effective dose comes from 10 mCi radioligand (= 0.38 / 2 rem) plus two whole body transmission scans (2 * 0.56 rem) for a total of 1.31 rem. This dose is described in the consent form as "about 2 rem."

Phase 2: Three brain scans. The effective dose comes from three injections of 20 mCi radioligand (3 * 0.38 rem) plus up to six head transmission scans (6 * 0.02 rem) for a total of 1.26 rem. This dose is described in the consent for as "about 1 rem."

Phase 3: One Whole Body Scan. The effective dose comes from one injection of 20 mCi radioligand (0.38 rem) plus up to two transmission scans (2 * 0.56 rem) for a total of 1.5 rem. This dose is also described in the consent form as "about 2 rem."

Phase 4: Two brain scans. The effective dose comes from two injections of 20 mCi radioligand (2 * 0.38 rem) plus up to four head transmission scans (4 * 0.02 rem) for a total of 0.84 rem. This dose is described in the consent for as "about 1 rem."

Each subject will not participate in more than one phase of this study.

c. PET scanning

PET scans, which detect injected radioactivity within the body, are not associated with any known physical hazards to the subject lying on the table. We routinely use a series of procedures to minimize the risk of discomfort during scanning sessions. Namely, the procedures are conducted in the presence of trained health professionals to whom participants will have ready access should they experience any problems. Participants can communicate with the

trained health professionals while in the scanner and can be removed from the scanner and withdraw from the study at any time if they wish to do so. Participants can also request that the operator stop the scan.

d. Arterial line placement

Placement of a radial arterial catheter may cause bruising or infection. There is also a risk of occlusion and microemboli. Over 3,000 arterial catheters have been placed to date at NIH Clinical Center for PET scans. Of these, only two complications requiring physician's care arose. In the first case, a small radial artery aneurysm developed several months later, which was successfully repaired surgically. In the second case, a radial artery thrombosis developed 28 days later, which was also successfully repaired surgically.

e. Intravenous line placement

Venous catheter insertion can be associated with discomfort, bruising, infection, or clot formation. Using proper placement techniques will minimize these risks.

In case of tracer extravasation, we will stop the study, remove the venous line from the arm, and apply cold compress to the site.

f. Blood sampling

Participants will have arterial and venous blood sampling. The total amount of blood drawn will not exceed 500 mL. Some of the blood will be collected via phlebotomy and some through venous and arterial lines. Blood sampling may lead to the formation of small subcutaneous hematomas caused by blood leaking from a punctured blood vessel. Such hematomas cause only minor discomfort. They are not dangerous and require no treatment other than reassuring the patient. There is also a small risk of infection at the site of the needle puncture, which can be readily treated with antibiotic therapy We will ask participants not to donate blood within 8 weeks prior to the study or for 8 weeks following the study.

g. MRI

People are at risk for injury from the MRI magnet if they have pacemakers or other implanted electrical devices, brain stimulators, some types of dental implants, aneurysm clips (metal clips on the wall of a large artery), metallic prostheses (including metal pins and rods, heart valves, and cochlear implants), permanent eyeliner, implanted delivery pump, or shrapnel fragments. Welders and metal workers are also at risk for injury because of possible small metal fragments in the eye of which they may be unaware. Subjects will be screened for these conditions before having any scan, and if they have any, they will not receive an MRI scan.

It is not known if MRI is completely safe for a developing fetus. Therefore, all women of childbearing potential will have a pregnancy test performed no more than 24 hours before each MRI scan. The scan will not be done if the pregnancy test is positive.

People with fear of confined spaces may become anxious during an MRI. Those with back problems may have back pain or discomfort from lying in the scanner. The noise from the scanner is loud enough to damage hearing, especially in people who already have hearing loss. Everyone having a research MRI scan will be fitted with hearing protection. Subjects will be asked to complete an MRI screening form for each MRI scan they have. There are no known long-term risks associated with MRI scans.

h. BPN14770 administration

Participants in Phase 2 will receive BPN14770 50 mg BID for three days, and a single dose of BPN14770 on the fourth day. Only minor adverse events are expected, which include: headache, diarrhea, nausea, vomiting, hot flashes, increased alanine aminotransferase and aspartate aminotransferase, hypotension, and dizziness.

8. Subject Safety Monitoring

Participants will be carefully monitored by clinical staff and asked about the presence of adverse events throughout the study, including during the PET procedure. The site of the catheter insertion will be carefully monitored for signs of bleeding.

a. Parameters to be monitored

For each ^{11}C-T-1650 scan: Pulse rate, blood pressure, respiratory rate, and EKG (either 3- or 12-lead) will be recorded within three hours before tracer injection and again at about 15, 30, and 120 minutes after injection. Laboratory tests will be done before and after tracer administration to monitor for changes. Monitoring will be performed by a credentialed clinician.

For BPN14770 (Phase 2): On the day of the first dose of BPN14770, before the dose as well as after the PET scan, the following lab tests will be conducted: CBC; acute care panel; hepatic panel; mineral panel; urinalysis; lipid panel; total protein; uric acid; creatine kinase; cholesterol; thyroid panel.

In addition, 3 -7 days after the last dose of BPN14770, participants are asked to visit NIH to check the presence of adverse events. The following parameters will be monitored during the follow up visit:

- Brief physical examination
- Orthostatic (supine sitting, standing) blood pressure and pulse
- ECG
- Body weight.
- CBC; acute care panel; hepatic panel; mineral panel; urinalysis; urine pregnancy test (females); and lipid panel; total protein; uric acid; creatine kinase; cholesterol; thyroid panel.

b. Criteria for individual subject withdrawal

Patients will be removed from the study if they become pregnant, are unable to continue to cooperate, or have an alteration in clinical status needing medical intervention. Patients may withdraw from the trial at any time at their own request. The investigators may withdraw a subject if the subject cannot meet study requirements, or the investigator deems that it is not in the subjects' best interest to continue.

9. Outcome Measures

a. Primary outcome measures

In baseline scans of ^{11}C-T-1650, the primary outcome measure is its binding, specifically identifiability, stability over time, and retest variability of V_T calculated with compartmental modeling. Phase 2 (BPN14770 administration) has two primary outcome measures, V_{ND} of ^{11}C-T-1650 and binding site occupancy of BPN14770. V_{ND} of ^{11}C-T-1650 will be measured by comparing ^{11}C-T-1650 binding (V_T) between Scan #1 (baseline) and Scan #2 because arterial

data will be available to calculate V_T. Binding site occupancy of BPN14770 under stable plasma concentrations of BPN14770 and V_{ND} of ^{11}C-T-1650 will be measured by comparing Scan #1 and #3 using brain uptake (SUV) normalized to venous concentrations of ^{11}C-T-1650 because arterial line will not be placed for Scan #3 due to the short interval of the scans.

b. Secondary outcome measures

Biodistribution and dosimetry of ^{11}C-T-1650.

10. Statistical Analysis

a. Analysis of data/ study outcomes

Brain imaging data will be analyzed with compartmental modeling using a nonlinear least squares analysis, graphical analysis of integration plot, and multilinear regression analysis. PET and MRI scans will be co-registered for anatomic definition of regions of interest. SUV will be calculated in various brain regions. Parametric images will be created using PMOD software (PMOD Technologies Ltd., Zurich, Switzerland

b. Power analysis

This kinetic modeling will give us ^{11}C-T-1650 binding values as well as their identifiability and stability over time. Because of inter-subject variability in pharmacokinetics and metabolism of PET radioligands, a sample size of approximately 12 is required in such studies [19-21]. A similar or a little smaller sample size of 10 is often used to measure drug occupancy by PET [22]. In order to account for drop-outs, we request a recruitment ceiling of 15 subjects for each of Phase 2 and 4. To estimate radiation absorbed doses by performing whole body imaging, a sample size of about eight is used in many studies. In order to account for drop-outs, we request a recruitment ceiling of 10 for whole body imaging (Phase 1 and 3 combined).

11. Human Subjects Protection

a. Subject selection

Participants will be selected based on the study's eligibility criteria. Participants will be admitted to the protocol regardless of gender, race, or ethnicity. NIMH employees/staff and their immediate family members will be excluded from the study per NIMH policy.

b. Justification for exclusion of children

Because this protocol has more than minimal risk from radiation exposure without possibility of direct benefit, inclusion of children is not appropriate.

c. Justification for the exclusion of vulnerable subjects

Pregnant women will be excluded because this protocol involves exposure to ionizing radiation. Lactating women will be excluded because radioisotopes may be excreted in milk.

Persons with brain disease and HIV infection are excluded to ensure that the results we see are related only to PDE4D and not to another abnormality in the brain, such as diseases that are likely to be associated with a disruption of the blood-brain barrier or a viral infection of the brain.

d. Justification for sensitive procedures

Not applicable

e. Safeguards for vulnerable populations

Pregnancy testing will be performed before PET and MRI scanning for any participants of child-bearing potential. Per the protocol eligibility criteria, if participants are able to become pregnant or father a child, adequate measures to prevent pregnancy must be in place. If participants chose to use barrier methods of contraception, the acceptable forms include diaphragm, cervical cap, male condom, female condom, and spermicidal foam and sponges. Females who say they are post-menopausal will have this status confirmed by a follicle stimulating hormone (FSH) test at Screening. In addition, all females must have a negative pregnancy test within 24 hours before the first dose of BPN14770 regardless of childbearing potential.

Protections for NIH employees and staff participating in this study include: 1) ensuring that participation or refusal to participate will have no effect, either beneficial or adverse, on the subject's employment or position at the NIH, 2) giving employees and staff who are interested in participating the "NIH Information Sheet on Employee Research Participation" prior to obtaining consent, and 3) ensuring that there will be no direct solicitation of employees or staff.

This study collects sensitive information. For example, we may collect information on drug and alcohol use, medical history and diagnoses. The PI will train study staff regarding obtaining and handling potentially sensitive and private information about co-workers through staff discussions and written branch/section procedures.

Patient advocate

A patients' rights representative is available to participants on this protocol. The representative is located in Building 10 and can be reached by phone at 301-496-2626. Participants may ask any questions about the study and may withdraw their consent at any time.

f. Qualifications of investigators

Robert B. Innis, MD, PhD – Branch Chief, Molecular Imaging Branch, NIMH/NIH. Dr. Innis has more than 25 years' experience using nuclear imaging. He is a clinical authorized user of radioisotopes and a credentialed physician. His responsibilities include study design, recruiting healthy subjects, consenting subjects, participating in PET scanning procedures, data analyses, and interpretation and publication of study results.

Milalynn S. Victorino, CRNP has been a family nurse practitioner since 2016 and a registered nurse since 2002. She is a credentialed clinician with prescriptive authority at the NIH clinical center. Her responsibilities include screening healthy subjects for research protocols, performing history and physicals with subject interviews, and consenting potential research subjects. She will also participate in PET scan procedures.

Ms. Desiree Ferraris Araneta, CNRP is a credentialed nurse practitioner. Ms. Ferraris Araneta has been a nurse practitioner for more than 15 years and was a nurse prior to that. She is a credentialed clinician at the NIH Clinical Center and has patient care credentials including prescriptive authority from the NIH Clinical Center. Her responsibilities include patient evaluation and care, consenting subjects, and participating in PET scan procedures.

Dr. Denise Rallis-Frutos, DNP is a credentialed nurse. Dr. Rallis-Frutos is a doctoral research nurse specialist and has been an associate investigator with the NIMH since 1996. Her responsibilities include patient evaluation and care, consenting subjects, and participating in PET and MRI scanning procedures.

Lora D. Weidner, PhD – Postdoctoral fellow, Molecular Imaging Branch NIMH/NIH. Dr. Weidner has her PhD in neuroscience and has 2 years of experience in PET studies at the University of Pittsburgh. There she gained experience in the analytical as well as the clinical aspects of PET, the latter involving patient interaction. At MIB, her responsibilities include, but are not limited to: study design, clinical evaluation of subjects, participating in PET scanning procedures, data analyses, and interpretation and publication of study results. Dr. Weidner will not obtain informed consent.

The Principal Investigator has verified that all individuals working on this protocol required to take HRPP training under OHSRP SOP 25 (Training requirements for the NIH Human Research Protections Program) have completed all required training.

The following study investigators will obtain informed consent: Dr. Innis, Dr. Rallis-Frutos, Ms. Ferraris Araneta, Ms. Victorino. Study investigators obtaining informed consent have completed the NIMH HSPU "Elements of Successful Informed Consent" training.

12. Anticipated Benefit

This study offers no direct benefit to participants, but is likely to yield generalizable knowledge about the density of PDE4D in the brains of healthy volunteers.

13. Classification of Risk

a. For adults

This study entails more than minimal risk with no prospect of direct benefit to participants.

b. For adults without consent capacity

Not applicable.

c. For children

Not applicable.

d. Overall risk and benefit consideration

The risks are reasonable in relation to the anticipated benefit.

14. Consent Documents and Process

a. Designation of those obtaining consent

Study investigators designated as able to obtain consent in section 11f above will obtain informed consent. All study investigators obtaining informed consent have completed the NIMH HSPU 'Elements of Successful Informed Consent' training. Co-workers will not consent each other.

b. Consent procedures

All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions regarding this study prior to signing.

c. Consent documents

The consent forms contain all required elements. Each of the four Phases has a separate consent form.

15. Data and Safety Monitoring

a. Data and safety monitor

This study will be monitored by an independent safety monitor (ISM): Dr. Carlos A. Zarate Jr., MD. Dr. Zarate is Chief of the Experimental Therapeutics and Pathophysiology Branch and Mood and Anxiety Disorders Research at the NIMH. Dr. Zarate is an internationally recognized leader in the clinical neuroscience and experimental therapeutics of mood and anxiety disorders, especially the use of glutamatergic agents like ketamine and riluzole in treatment-resistant unipolar and bipolar depression. He was senior author on one of the first studies demonstrating the relationship between ketamine infusion and reductions in suicidal thoughts, and has since published over 10 articles related to the neurobiology of suicide risk. He has a strong track record in recruiting, studying and publishing novel research findings and mentoring junior colleagues in his area of expertise. His primary responsibility will be to provide independent safety monitoring in a timely fashion.

b. Data and safety monitoring plan

About every six months, the PI will prepare for the Independent Monitor a report on data and safety parameters, including all AEs, SAEs, and protocol deviations. The Independent monitor will provide a written monitoring report to be submitted to the IRB at the time of continuing review.

c. Criteria for stopping the study or suspending enrollment or procedures

The study will be stopped in the event of a Serious Adverse Event related to research or if new data sheds light on the danger of any procedure used on this protocol. The PI/Independent Monitor and IRB will determine if changes are needed for the research to continue or if it will be closed. Any changes required as conditions for resuming the research must be submitted as an amendment and IRB-approved before the changes can be implemented.

16. Quality Assurance

a.QA Monitor

Quality assurance will be monitored by the PI, the research team, and the Office of Regulatory Oversight (ORO).

b. QA Plan

ORO monitors intramural research studies to ensure compliance with GCP, organizational policies and regulations. Audit frequency is determined by the ORO SOP based on the study level of risk. Results of the ORO audits are provided to the PI, the Clinical Director and the CNS

IRB. As an IND study, this protocol will be subject to GCP audits at study initiation and after the first enrolled subject. Timing of subsequent review will be established by the ORO but no less frequent than every other year.

17. Reporting of Unanticipated Problems, Adverse Events, and Protocol Deviations

Reporting of Unanticipated problems, adverse events and protocol deviations

The Principal Investigator is responsible for detecting, documenting, and reporting unanticipated problems, adverse events (AEs), including serious adverse events (SAEs), and deviations in accordance with NIH policy, IRB requirements, and federal regulations. Relatedness to the research of all serious adverse events will be determined by the PI in consultation with the CD.

Serious unanticipated problems, serious adverse events (including deaths) that are not unanticipated problems, and serious protocol deviations will be reported to the IRB and CD as soon as possible and in writing not more than 7 days after the PI first learns of the event, unless immediate reporting is waived for specific serious adverse events as noted below. Not serious unanticipated problems and not serious deviations will be reported to the IRB and CD as soon as possible and in writing not more than 14 days after the PI first learns of the event. Written reports will be submitted in PTMS.

All adverse events, deviations, and unanticipated problems will be summarized and reported at the time of Continuing Review.

It is anticipated that participants in this study will occasionally miss or fail to complete an assessment or procedure, such as a completion of a rating scale or a blood draw, or fail to complete a procedure or visit within protocol-specified time frames. Omissions such as these will be considered expected events and not protocol deviations provided they are infrequent and do not include data needed to assess safety or the primary study outcome. Cumulative proportions of these missed events in the study population will be presented to the IRB annually. In addition, the rate of omissions will be monitored by the Investigators. If an individual misses more than 15% of the required assessments/procedures or if more than 15% of the participants miss completion of the same assessment or procedure, it will be considered a deviation and a deviation report will be sent to the IRB within two weeks.

If the total number of expected items (study visits, study assessments/procedures) is less than 16, then two or more missed items are reportable. If the total number of expected items is greater than 16, then, if more than 15% are missed, it is reportable.

For ^{11}C-T1650 and BPN14770 Studied under IND

The PI will immediately report SAEs to the Sponsor according to the requirements of 21 CFR 312.64(b) and as agreed upon with the sponsor. The PI will record nonserious AEs and report them to the Sponsor within 14 days.

18. Alternatives to Participation

Subjects do not receive any treatment in this study or forego any treatment in order to participate in this study. The alternative, therefore, is not to participate.

19. Privacy

All research activities will be conducted in as private a setting as possible

20. Confidentiality

a. For research data and investigator medical records

Data will be kept in password-protected computers. Only study investigators will have access to the data. This study collects sensitive medical information. The PI will train study staff regarding obtaining and handling potentially sensitive and private information about co-workers through staff discussions and written branch/section procedures.

b. For stored samples

Blood samples to measure ^{11}C-T1650 concentrations will be discarded at the end of the PET scan. Blood samples to measure BPN14770 concentrations will be kept in a locked freezer of MIB/NIMH in the NIH Bethesda campus. Data will be stored using codes that we assign.

c. Special precautions

Data will be kept in password-protected computers. Only study investigators will have access to the data.

21. Conflict of Interest

a. Distribution of NIH guidelines

NIH guidelines on conflict of interest have been distributed to all investigators.

b. Conflict of interest

There are no conflicts of interest to report.

c. Role of a commercial company or sponsor

Tetra Discovery Partners has developed and is providing a new PDE4D selective inhibitor BPN14770 and the precursor to synthesize ^{11}C-labeled PET ligand, ^{11}C-T-1650 to NIMH without charge. Tetra Discovery Partners will receive information learned in the study, but they will not receive any identifying information about the participants. No NIMH investigator involved in this study receives any payment or other benefits from Tetra Discovery Partners.

Tetra Discovery Partners intends to supply BPN14770 and ^{11}C-T-1650 and NIMH will finance all aspects of the study. The subjects will not bear responsibility for any costs associated with study participation

22. Technology Transfer

CTA 2018-0122 with Tetra Discovery Partners has been uploaded in PTMS.

23. Research and Travel Compensation

Volunteers will be compensated for time and research-related inconveniences. Reimbursement is based on NIH standards for time devoted to the research project. Participants will be paid for each portion of the study they have completed whether or not they opt for early withdrawal from participation. Without any delay of study procedures or unanticipated inconvenience, the maximum possible compensation is $940. If the investigators need to delay

study procedures or if additional time is needed for completion, subjects may receive additional compensation in accordance with NIH guidelines.

Employees and staff who participate during work hours must have permission from their supervisor. NIH employees must either participate outside of work hours or take leave in order to receive compensation.

Subjects undergoing whole body scan (Phase1 and 3)

Visit 1 to NIH	
PET scanning	$150
Antecubital venous catheters	$30
Pregnancy test	$10
Movement restriction	$10
Total	$200

Subjects Enzyme occupancy brain scans (Phase 2)

	Total Pay
Visit 1 to NIH	
MRI	$100
Pregnancy test	$10
Visit 2 to NIH	
PET scanning	$150
Arterial catheter	$60
Antecubital venous catheters	$30
Pregnancy test	$10
Movement restriction	$10
Visit 3 to NIH	
PET scanning	$150
Arterial catheter	$60
Antecubital venous catheters	$30
Pregnancy test	$10
Movement restriction	$10
BPN14770 administration	$50
Visit 4 to NIH	
PET scanning	$150
Arterial catheter	$60
Antecubital venous catheters	$30
Pregnancy test	$10
Movement restriction	$10
Total	$940

Subjects undergoing Test Retest Brain Scans (Phase 4)

	Total Pay
Visit 1 to NIH	
MRI	$100
Pregnancy test	$10
Visit 2 to NIH	
PET scanning	$150
Arterial catheter	$60
Antecubital venous catheters	$30
Pregnancy test	$10
Movement restriction	$10
Visit 3 to NIH	
PET scanning	$150
Arterial catheter	$60
Antecubital venous catheters	$30
Pregnancy test	$10
Movement restriction	$10
Total	$630

NIH will cover travel expenses to the Clinical Center for all participants in accord with NIH guidelines.

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25. Attachments

- List of P450 inhibitors

26. Consent Forms

- Phase 1: For the first healthy subject who has a whole body scan
- Phase 2: Healthy subjects who have three brain scans
- Phase 3: Additional healthy subjects who have a whole body scan
- Phase 4: Healthy subjects who have two brain scans