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Official Title: Vitamin D as a Therapeutic Adjunct in the Stimulant Treatment of ADHD: a Proof-of-concept Study of Stimulant-induced Dopamine Release Using [11C]-PHNO PET in Healthy Humans

Document Type: Protocol w SAP

Date: 11/1/2023

YALE UNIVERSITY
HUMAN INVESTIGATION COMMITTEE

Application to Involve Human Subjects in Biomedical Research
100 FR1 (2016-1)

SECTION I: ADMINISTRATIVE INFORMATION

Title of Research Project: Vitamin D as a therapeutic adjunct in the stimulant treatment of ADHD: a proof-of-concept study of stimulant-induced dopamine release using [^{11}C]-PHNO PET in healthy humans			
Principal Investigator: Marc Potenza, MD, PhD		**Yale Academic Appointment:** Professor	
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Protocol Correspondent Name & Address (*if different than PI*): Jessica Costeines, MSW			
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Yale Cancer Center CTO Protocol Correspondent Name & Address (*if applicable*):			
Campus Phone:	**Fax:**	**E-mail:**	
Business Manager:			
Campus Phone :	**Fax :**	**E-mail**	

Faculty Advisor:(required if PI is a student, resident, fellow or other trainee) X **NA**		**Yale Academic Appointment:**	
Campus Address:			
Campus Phone:	**Fax:**	**Pager:**	**E-mail:**

Investigator Interests:

Does the principal investigator, or do any research personnel who are responsible for the design, conduct or reporting of this project or any of their family members (spouse or dependent child) have an incentive or interest, financial or otherwise, that may affect the protection of the human subjects involved in this project, the scientific objectivity of the research or its integrity? Note: The Principal Investigator (Project Director), upon consideration of the individual's role and degree of independence in carrying out the work, will determine who is responsible for the design, conduct, or reporting of the research.

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See Disclosures and Management of Personal Interests in Human Research
http://www.yale.edu/hrpp/policies/index.html#COI
☐ Yes ☒ No

Do you or does anyone on the research team who is determined by you to be responsible for the design, conduct or reporting of this research have any patent (sole right to make, use or sell an invention) or copyright (exclusive rights to an original work) interests related to this research protocol?
☐ Yes ☒ No

If yes to either question above, list names of the investigator or responsible person:

The Yale University Principal Investigator, all Yale University co-investigators, and all Yale University individuals who are responsible for the design, conduct or reporting of research must have a current financial disclosure form on file with the University's Conflict of Interest Office. Yale New Haven Hospital personnel who are listed as co-investigators on a protocol with a Yale University Principal Investigator must also have a current financial disclosure form on file with the University's Conflict of Interest Office. If this has not been done, the individual(s) should follow this link to the COI Office Website to complete the form: http://www.yale.edu/coi/

NOTE: The requirement for maintaining a current disclosure form on file with the University's Conflict of Interest Office extends primarily to Yale University and Yale-New Haven Hospital personnel. **Whether or not they are required to maintain a disclosure form with the University's Conflict of Interest Office, all investigators and individuals deemed otherwise responsible by the PI who are listed on the protocol are required to disclose to the PI any interests that are specific to this protocol.**

SECTION II: GENERAL INFORMATION

1. **Performing Organizations:** Identify the hospital, in-patient or outpatient facility, school or other agency that will serve as the location of the research. Choose all that apply:

a. Internal Location[s] of the Study:

☒ Magnetic Resonance Research Center (MR-TAC)
☐ Yale Cancer Center/Clinical Trials Office (CTO)
☐ Yale Cancer Center/Smilow
☐ Yale-New Haven Hospital
☐ Cancer Data Repository/Tumor Registry
☐ Specify Other Yale Location:
☒ Yale University PET Center
☐ YCCI/Church Street Research Unit (CSRU)
☐ YCCI/Hospital Research Unit (HRU)
☐ YCCI/Keck Laboratories
☐ Yale-New Haven Hospital—Saint Raphael Campus

b. External Location[s]:

☐ APT Foundation, Inc.
☐ Haskins Laboratories

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☒ Connecticut Mental Health Center
☒ Clinical Neuroscience Research Unit (CNRU)
☐ Other Locations, Specify:

☐ John B. Pierce Laboratory, Inc.
☐ Veterans Affairs Hospital, West Haven
☐ International Research Site
(Specify location(s)):

c. Additional Required Documents *(check all that apply)*: ☐ N/A

☐ *YCCI-Scientific and Safety Committee (YCCI-SSC) Approval Date:
☐ *Pediatric Protocol Review Committee (PPRC) Approval Date:
☐ *YCC Protocol Review Committee (YRC-PRC) Approval Date:
☐ *Dept. of Veterans Affairs, West Haven VA HSS Approval Date:
☐ *Radioactive Drug Research Committee (RDRC) Approval Date:
☒ YNHH-Radiation Safety Committee (YNHH-RSC) Approval Date:
☒ Yale University RSC (YU-RSC) Approval Date:
☒ Magnetic Resonance Research Center PRC (MRRC-PRC) Approval Date:
☐ *Nursing Research Committee Approval Date:
☐ YSM/YNHH Cancer Data Repository (CaDR) Approval Date:
☐ Dept. of Lab Medicine request for services or specimens form
☐ Imaging on YNHH Diagnostic Radiology equipment request form (YDRCTO request) found at http://radiology.yale.edu/research/ClinTrials.aspx)

Approval from these committees is required before final HIC approval is granted. See instructions for documents required for initial submission and approval of the protocol. Allow sufficient time for these requests. Check with the oversight body for their time requirements.

2. **Probable Duration of Project:** State the expected duration of the project, including all follow-up and data analysis activities.

6 years

3. **Research Type/Phase: (Check all that apply)**

a. **Study Type**

☒ Single Center Study
☐ Multi-Center Study

Does the Yale PI serve as the PI of the multi-site study? Yes ☐ No X

☐ Coordinating Center/Data Management
☐ Other:

b. **Study Phase** ☐ **N/A**

☒ Pilot ☐ Phase I ☐ Phase II ☐ Phase III ☐ Phase IV
☐ Other (*Specify)*

4. **Area of Research: (Check all that apply)** Note that these are overlapping definitions and more than one category may apply to your research protocol. Definitions for the following can be found in the instructions section 4c:

☐ Clinical Research: Patient-Oriented ☐ Clinical Research: Outcomes and

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☐ Clinical Research: Epidemiologic and Behavioral
☒ Translational Research #1 ("Bench-to-Bedside")
☐ Translational Research #2 ("Bedside-to-Community")

Health Services
☐ Interdisciplinary Research
☐ Community-Based Research

5. Is this study a clinical trial? Yes ☒ No ☐

NOTE the current ICMJE (International Committee of Medical Journal Editors) definition of a clinical trial: "any research study that prospectively assigns human participants or groups of humans to one or more health-related interventions to evaluate the effects on health outcomes." Health-related interventions include any intervention used to modify a biomedical or health-related outcome (for example, drugs, surgical procedures, devices, behavioral treatments, dietary interventions, and process-of-care changes). Health outcomes include any biomedical or health-related measures obtained in patients or participants, including pharmacokinetic measures and adverse events"

If yes, where is it registered?

Clinical Trials.gov registry ☒
Other (*Specify*)

*Registration of clinical trials **at their initiation** is required by the FDA, NIH and by the ICMJE.*

If this study is registered on clinicaltrials.gov, there is new language in the consent form and compound authorization that should be used.

For more information on registering clinical trials, including whether your trial must be registered, see the YCCI webpage, http://ycci.yale.edu/researchers/ors/registerstudy.aspx or contact YCCI at 203.785.3482)

6. Does the Clinical Trials Agreement (CTA) require compliance with ICH GCP (E6)? Yes ☒ No ☐

7. Will this study have a billable service? *A billable service is defined as any service rendered to a study subject that, if he/she was not on a study, would normally generate a bill from either Yale-New Haven Hospital or Yale Medical Group to the patient or the patient's insurer. The service may or may not be performed by the research staff on your study, but may be provided by professionals within either Yale-New Haven Hospital or Yale Medical Group (examples include x-rays, MRIs, CT scans, specimens sent to central labs, or specimens sent to pathology). Notes: 1. There is no distinction made whether the service is paid for by the subject or their insurance (Standard of Care) or by the study's funding mechanism (Research Sponsored). 2. This generally includes new services or orders placed in EPIC for research subjects.*

Yes☒ No ☐

If answered, "yes", this study will need to be set up in OnCore, Yale's clinical research management system, for Epic to appropriately route research related charges. Please contact oncore.support@yale.edu

8.. Are there any procedures involved in this protocol that will be performed at YNHH or one of its affiliated entities? Yes ___ No __X_ *If Yes, please answer questions a through c and note instructions below. If No, proceed to Section III.*

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a. Does your YNHH privilege delineation currently include the **specific procedure** that you will perform?
b. Will you be using any new equipment or equipment that you have not used in the past for this procedure?

c. Will a novel approach using existing equipment be applied?

If you answered "no" to question 8a, or "yes" to question 8b or c, please contact the YNHH Department of Physician Services (688-2615) for prior approval before commencing with your research protocol.

Please note that if this protocol includes Yale-New Haven Hospital patients, including patients at the HRU, the Principal Investigator and any co-investigators who are physicians or mid-level practitioners (includes PAs, APRNs, psychologists and speech pathologists) who may have direct patient contact with patients on YNHH premises must have medical staff appointment and appropriate clinical privileges at YNHH. If you are uncertain whether the study personnel meet the criteria, please telephone the Physician Services Department at 203-688-2615. ***By signing this protocol as a PI, you attest that you and any co-investigator who may have patient contact has a medical staff appointment and appropriate clinical privileges at YNHH****.*

SECTION III: FUNDING, RESEARCH TEAM AND TRAINING

1. **Funding Source:** Indicate all of the funding source(s) for this study. Check all boxes that apply. Provide information regarding the external funding source. This information should include identification of the agency/sponsor, the funding mechanism (grant or contract), and whether the award is pending or has been awarded. Provide the M/C# and Agency name (if grant-funded). If the funding source associated with a protocol is "pending" at the time of the protocol submission to the HIC (as is the case for most NIH submissions), the PI should note "Pending" in the appropriate section of the protocol application, provide the M/C# and Agency name (if grant-funded) and further note that University (departmental) funds support the research (until such time that an award is made).

This information can be found in IRES IRB.

IRB Review fees are charged for projects funded by Industry or Other For-Profit Sponsors. Provide the Name and Address of the Sponsor Representative to whom the invoice should be sent. ***Note: the PI's home department will be billed if this information is not provided.***

Send IRB Review Fee Invoice To:
Name:
Company:
Address:

2. **Research Team:** List all members of the research team. Indicate under the affiliation column whether the investigators or study personnel are part of the Yale faculty or staff, or part of the faculty or staff from a collaborating institution, or are not formally affiliated with any institution. **ALL members of**

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the research team MUST complete Human Subject Protection Training (HSPT) and Health Insurance Portability and Accountability Act (HIPAA) Training before they may be listed on the protocol. See NOTE below.

NOTE: The HIC will remove from the protocol any personnel who have not completed required training. A personnel protocol amendment will need to be submitted when training is completed.

Please see IRES IRB for a complete list of study personnel.

SECTION IV: PRINCIPAL INVESTIGATOR/FACULTY ADVISOR/ DEPARTMENT CHAIR AGREEMENT

As the **principal investigator** of this research project, I certify that:

- The information provided in this application is complete and accurate.
- I assume full responsibility for the protection of human subjects and the proper conduct of the research.
- Subject safety will be of paramount concern, and every effort will be made to protect subjects' rights and welfare.
- The research will be performed according to ethical principles and in compliance with all federal, state and local laws, as well as institutional regulations and policies regarding the protection of human subjects.
- All members of the research team will be kept apprised of research goals.
- I will obtain approval for this research study and any subsequent revisions prior to my initiating the study or any change and I will obtain continuing approval of this study prior to the expiration date of any approval period.
- I will report to the HIC any serious injuries and/or other unanticipated problems involving risk to participants.
- I am in compliance with the requirements set by the University and qualify to serve as the principal investigator of this project or have acquired the appropriate approval from the Dean's Office or Office of the Provost, or the Human Subject Protection Administrator at Yale-New Haven Hospital, or have a faculty advisor.
- I will identify a qualified successor should I cease my role as principal investigator and facilitate a smooth transfer of investigator responsibilities.

_____ _____

PI Name (PRINT) and Signature Date

As the **faculty advisor** of this research project, I certify that:

- The information provided in this application is complete and accurate.
- This project has scientific value and merit and that the student or trainee investigator has the necessary resources to complete the project and achieve the aims.
- I will train the student investigator in matters of appropriate research compliance, protection of human subjects and proper conduct of research.
- The research will be performed according to ethical principles and in compliance with all federal, state and local laws, as well as institutional regulations and policies regarding the protection of human subjects.
- The student investigator will obtain approval for this research study and any subsequent revisions prior to initiating the study or revision and will obtain continuing approval prior to the expiration of any approval period.
- The student investigator will report to the HIC any serious injuries and/or other unanticipated problems involving risk to participants.
- I am in compliance with the requirements set forth by the University and qualify to serve as the faculty advisor of this project.
- I assume all of the roles and responsibilities of a Principal Investigator even though the student may be called a PI.

_____ _____

Advisor Name (PRINT) and Signature Date

Department Chair's Assurance Statement

Do you know of any real or apparent institutional conflict of interest (e.g., Yale ownership of a sponsoring company, patents, licensure) associated with this research project?

☐ Yes (provide a description of that interest in a separate letter addressed to the HIC.)
☐ No

As Chair, do you have any real or apparent protocol-specific conflict of interest between yourself and the sponsor of the research project, or its competitor or any interest in any intervention and/or method tested in the project that might compromise this research project?

☐ Yes (provide a description of that interest in a separate letter addressed to the HIC)
☐ No

I assure the HIC that the principal investigator and all members of the research team are qualified by education, training, licensure and/or experience to assume participation in the conduct of this research trial. I also assure that the principal investigator has departmental support and sufficient resources to conduct this trial appropriately.

_____ _____

Chair Name (PRINT) and Signature Date

_____

Department

YNHH Human Subjects Protection Administrator Assurance Statement

Required when the study is conducted solely at YNHH by YNHH health care providers.

As Human Subject Protection Administrator (HSPA) for YNHH, I certify that:

- I have read a copy of the protocol and approve it being conducted at YNHH.
- I agree to notify the IRB if I am aware of any real or apparent institutional conflict of interest.
- The principal investigator of this study is qualified to serve as P.I. and has the support of the hospital for this research project.

_____ _____

YNHH HSPA Name (PRINT) and Signature Date

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SECTION V: RESEARCH PLAN

1. **Statement of Purpose:** State the scientific aim(s) of the study, or the hypotheses to be tested. **Specific Aim 1:** As part of a within-subject, two-days, study design, to determine whether acute calcitriol (vs. placebo) pre-treatment is associated with greater amphetamine (Amp)-induced dopamine (DA) release in the caudate, putamen, ventral striatum (VST), and substantia nigra / ventral tegmental area (SN/VTA) of healthy human subjects. **Hypothesis:** Given prior evidence of increased amphetamine-evoked DA release in rats pretreated with calcitriol compared to placebo (Cass, Peters et al. 2012), we hypothesize that pre-treatment with calcitriol will produce greater Amp-induced DA release relative to placebo, resulting in lower post-Amp, **[^{11}C]-PHNO** BP_{ND} values on calcitriol as compared to placebo days. If confirmed, such data would provide compelling preliminary support for the adjunctive use of calcitriol for enhancing stimulant medication effects (leading to larger scale PET imaging and/or clinical trial studies in humans with Attention Deficit Hyperactivity Disorder or ADHD).

   Specific Aim 2: To determine whether acute calcitriol (vs. placebo) pre-treatment is associated with better performance on a test of attention (e.g., the continuous Performance Task or CPT–IP), after treatment with amphetamine. **Hypothesis**: We hypothesize that healthy human subjects pre-treated with calcitriol will have faster reaction times/higher accuracy on the CPT-IP vs. subjects pre-treated with placebo, after treatment with amphetamine.

   Specific Aim 3: As part of a within-subject, two-days, study design, to determine whether acute calcitriol (vs. placebo) pre-treatment is associated with greater amphetamine (Amp)-induced dopamine (DA) release in the caudate, putamen, ventral striatum (VST), and substantia nigra / ventral tegmental area (SN/VTA) of subjects with attention deficit hyperactivity disorder (ADHD). **Hypothesis:** Similar to our hypothesis with healthy controls and congruent with our preliminary findings, we hypothesize that pre-treatment with calcitriol will produce greater Amp-induced DA release relative to placebo, resulting in lower post-Amp, **[^{11}C]-PHNO** BP_{ND} values on calcitriol as compared to placebo days.

   Specific Aim 4: To determine whether acute calcitriol (vs. placebo) pre-treatment is associated with better performance on a test of attention (e.g., the continuous Performance Task or CPT–IP), after treatment with amphetamine. **Hypothesis**: Similar to our hypothesis with healthy controls and congruent with our preliminary findings, we hypothesize that individuals with ADHD pre-treated with calcitriol will have faster reaction times/higher accuracy on the CPT-IP vs. subjects pre-treated with placebo, after treatment with amphetamine

2. **Background:** Describe the background information that led to the plan for this project. Provide references to support the expectation of obtaining useful scientific data.

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Attention deficit hyperactivity disorder (ADHD) is a common and serious neuropsychiatric disorder in children. With a prevalence of ~ 5 % in school-age children, ADHD is responsible for a disproportionate (i.e., 30 - 50 %) of referrals to mental health services (MTA 1999, Wilens, Adler et al. 2008). ADHD has considerable consequences, making it one of the largest public health problems in the US (Biederman, Faraone et al. 1993, Biederman, Petty et al. 2008, Klein, Mannuzza et al. 2012). For example, work difficulties, educational/occupational under-attainment (Biederman, Petty et al. 2008), short-lived relationships/risky sexual behaviors (Biederman, Petty et al. 2008) (Biederman, Faraone et al. 1993, Kooij, Bejerot et al. 2010), criminality (Biederman, Petty et al. 2008), automobile collisions (Barkley, Murphy et al. 2002), and higher rates of lifetime mood lability (Biederman, Petty et al. 2008) are all associated with the disorder.

Although the neurobiological basis of ADHD is unknown, it has been postulated that there is a dysfunction in catecholamines (e.g., norepinephrine and dopamine [DA]) resulting in dysregulation of fronto – cortical and striatal structures. For instance, DA has been associated with attention, salience (Volkow, Wang et al. 2004), focus, vigilance, and on-task behavior (Wilens and Dodson 2004). Stimulants block the presynaptic reuptake of DA (e.g., methylphenidate [Mph] and amphetamines [Amp]) and enhance its presynaptic release (e.g., Amp), making them some of the most commonly prescribed medications for the treatment of adult ADHD (Wilens, Faraone et al. 2004) (Solanto 1998, Volkow, Wang et al. 1998). For instance, a Positron Emission Tomography (PET) study showed how Mph's effects on extracellular DA in the striatum is associated with higher interest/motivation in a mathematical task (Volkow, Wang et al. 2004). Notwithstanding the fact that stimulants are the most commonly prescribed medications for the disorder (Solanto 1998, Wilens and Dodson 2004, Wilens, Faraone et al. 2004) and the increasing evidence documenting their safety in the treatment of ADHD, parents nonetheless face significant uncertainty, fear and resistance when it comes starting such medications in their child (Brinkman, Sherman et al. 2009). For example, many children develop side effects unrelated to the therapeutic effects of the drugs, necessitating discontinuation, and still others will fail to respond adequately. In addition, there have been increasing concerns about diversion of stimulants, with lifetime rates as high as 16 to 29 % (Wilens, Adler et al. 2008).

As a result, ***there is a considerable interest in / need for strategies aimed at minimizing stimulant exposure / enhancing stimulant benefit in ADHD children***.

The Vitamin D precursor, 7-dehydrocholesterol, is a cholesterol derivative located in the skin. Exposure to ultraviolet light and spontaneous isomerization convert it to cholecalciferol (Holick 1995). Then, two hydroxylation steps first in the liver and then in the kidney make 1, 25 $(OH)_2D$ (dihyrdoxycholecalciferol or calcitriol) – i.e. the active form of vitamin D (Holick 2007). The rate limiting enzyme in this activation process, 1α hydroxylase, was primarily found to be in kidney tubules (Zehnder and Hewison 1999), but we now know that this enzyme

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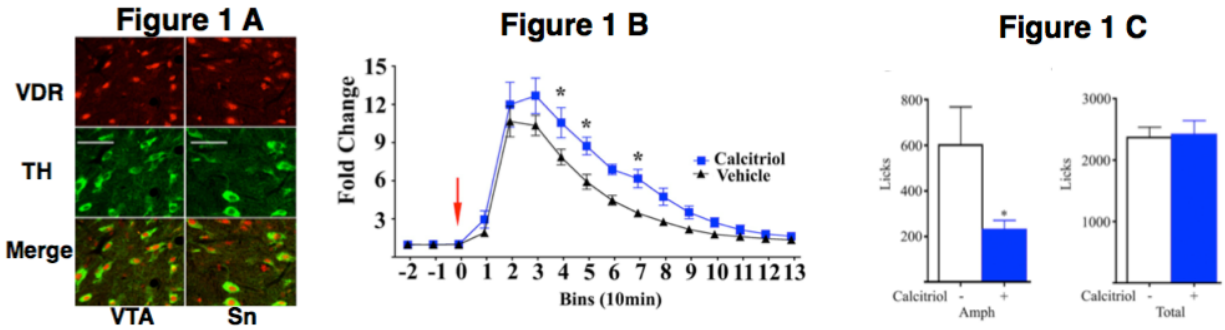
is expressed in many different cells, including neurons (Stumpf, Sar et al. 1979, Bouillon, Carmeliet et al. 2008, Buell and Dawson-Hughes 2008). The vitamin D receptor (VDR) is present in DA neurons (Cui, Pelekanos et al. 2013) and our colleagues/collaborators (DiLeone et al.) have confirmed its cellular co-localization with tyrosine hydroxylase (TH) (Fig 1A), the rate-limiting enzyme in DA synthesis. Intriguingly, and consistent with subchronic dosing studies (Cass, Peters et al. 2012), they have shown that a single dose of calcitriol vs. vehicle resulted in significantly enhanced Amp-induced DA release as measured by both *in vivo* microdialysis (Fig 1B) and Fast Scan Cyclic Voltammetry (FSCV). Moreover, this same calcitriol dose resulted in reductions in Amp consumption (e.g., number of licks) in rodents (Figure 1C; left) without affecting total intake of fluid (Figure 1C; right). Taken together, these data demonstrate that the biologically active form of Vitamin D (i.e., calcitriol) enhances stimulant-induced DA release and produces changes in behavior consistent with enhanced stimulant effects in rodents.



A Causal and/or Contributory Role for Vitamin D in ADHD: the jury is out. Increases in the rates of childhood ADHD over the past two decades have lead to speculation that calcitriol deficiency (e.g., secondary to the increased use of sunscreen and/or increases in sedentary, indoor lifestyles in children) plays a causal/contributory role in the etiology of ADHD (Arns, van der Heijden et al. 2013). To date, evidence of a direct link is lacking. One study showed higher maternal circulating Vitamin D levels in pregnancy are associated with lower risk of developing ADHD-like symptoms in childhood (Morales, Julvez et al. 2015). On the other hand, another study did not replicate the above association (Strom, Halldorsson et al. 2014), and a prospective study using umbilical cord samples stored at the time of birth reported no difference in serum vitamin D levels between ADHD group versus healthy controls (Gustafsson, Rylander et al. 2015). In terms of clinical trials, one randomized double blind study among adults with ADHD reported a beneficial effect of the intervention, measured with the Conners Adult ADHR rating scale, in comparison with placebo, but the intervention included the combination of vitamin D and several other micronutrients (Rucklidge, Frampton et al. 2014). An analysis of moderators of a positive response to ADHD behaviors did not reveal a significant predictive effect of vitamin D (Rucklidge, Johnstone et al. 2014).

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However, recent studies provide intriguing indirect evidence of an inverse relationship between solar intensity (SI) and/or altitude (a proxy for greater sun/UV light exposure) and regional rates of ADHD. One study examined three large datasets across 49 U.S. states for 2003 and 2007, and across 9 non-U.S. countries. This study examined the prevalence of ADHD and Solar Intensity (SI) maps. They found an inverse association between solar intensity and prevalence of ADHD (Arns, van der Heijden et al. 2013). Another study examined two national survey datasets. They found an inverse relationship between altitude and prevalence of ADHD (Huber, Kim et al. 2015). We hypothesize, as suggested by Huber, that a common denominator on the above studies is the increased vitamin D levels in those exposed to a higher solar intensity, which is known to increase with altitude (Arns, Swanson et al. 2015).

Positron Emission Tomography (PET) can be used to measure stimulant-induced DA release in humans. PET is a non-invasive, brain imaging technique in which radioactively labeled medications are used measure drug targets (e.g., receptors) and related neurochemistry *in vivo*.

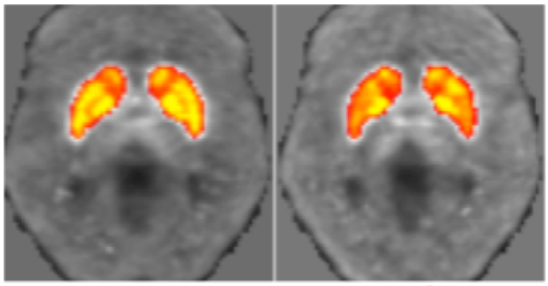

Figure 2. Representative striatal [^{11}C](+) PHNO parametric BP_{ND} images pre (left) and post (right) amphetamine administration courtesy of Kelly P. Cosgrove, PhD.

For example, work over the past two decades has employed the antagonist radioligand, [^{11}C]-raclopride, to not only measure $D_{2/3}$ receptors in the brain, but also the release of its endogenous neurotransmitter (i.e., DA) following stimulant medication administration (i.e., by comparing the relative displacement of radiotracer in comparison to placebo) (Cardenas, Houle et al. 2004). In fact, such methods have been employed previously to measure DA release in adults with ADHD (Volkow, Wang et al. 2007) (i.e., which was positively associated with improvement in ADHD symptoms, such as inattention) (Volkow, Wang et al. 2007). Our group has considerable experience conducting such experiments with dextro-ampethamine (at doses in excess of those employed here; e.g., 0.4 mg/kg vs. 0.3 mg/kg). At the Yale PET center, we have characterized and applied an improved agonist radiotracer for the D2/3 receptor, [^{11}C]-PHNO, demonstrating its excellent test-retest variability in striatum (Gallezot, Zheng et al. 2014) and its greater sensitivity to Amp-induced DA release relative to traditional antagonist ligands (i.e., [^{11}C]-raclopride) (Shotbolt, Tziortzi et al. 2012). As such, [^{11}C]-PHNO constitutes an optimal tracer for our proposed studies evaluating the effects of calcitriol on stimulant-induced DA release in humans. We have experience conducting these experiments with [^{11}C]-raclopride as well as with agonist ligands, such as [^{11}C]-PHNO, in humans (Fig 2).

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Clinical evidence of calcitriol's capacity to enhance stimulant-induced DA release in healthy humans as measured by ^{11}C-PHNO PET: We have obtained ^{11}C-PHNO PET data in two healthy males (28 ± 1 yrs) who successfully completed two sets of paired, same-day, pre- (baseline) and post-AMPH scans of subcortical $D_{2/3}$ receptor availability (BP_{ND}) on both active calcitriol (3.0 µg) and placebo pretreatment days one week apart (N=4 scans/subject)(**Fig 2A left; see next page**). In contrast to preclinical (rodent mRNA) findings, no effects of calcitriol on baseline measures of $D_{2/3}$ receptor availability vs. placebo were observed (mean ± SD BP_{ND} = 4.5 ± 0.8 vs. 4.3 ± 0.3, respectively; **Fig 2A center**). However, as hypothesized, calcitriol produced robust increases in AMPH-stimulated DA release as measured by % change in pre- vs. post-AMPH BP_{ND} vs. placebo (ΔBP_{ND} = 25 ± 3% vs. 15 ± 2%; **Fig 2A right**). Calcitriol findings in VS extended to other subcortical regions as well (caudate, putamen, and pallidum). Importantly, calcitriol also enhanced vigilance/attention after AMPH as measured by the Continuous Performance Task-AX **(Fig 2B)**. Thus, PET measures of DA release and neurocognitive tests of vigilance/attention provide strong support for calcitriol's potential efficacy in humans.

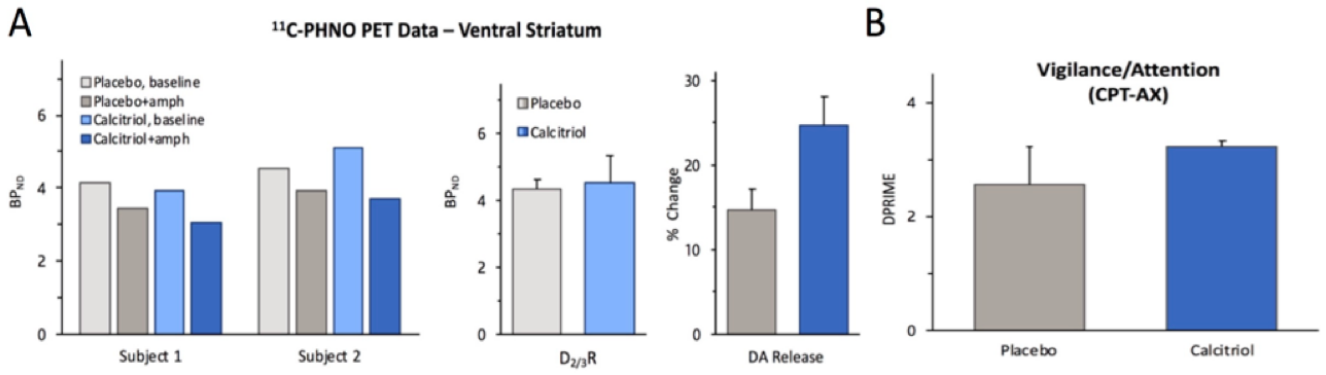

Figure 2A. (Left) Ventral striatal (i.e., nucleus accumbens) ^{11}C-PHNO measures of $D_{2/3}$ receptor availability (BP_{ND}) from four PET scans in each of two healthy subjects, including from paired baseline (light shades) and post-amphetamine scans (dark shades) on active calcitriol (blue) and placebo (grey) test days; **(Center)** Similar baseline $D_{2/3}$ receptor availability (mean ± SD BP_{ND}) in subjects after calcitriol (blue) vs. placebo (grey) administration; and **(Right)** Enhanced amphetamine-stimulated dopamine (DA) release as evidenced by greater ΔBP_{ND} (mean ± SD % change = 100 x [BP_{ND} pre-amphetamine – BP_{ND} post-amphetamine] / BP_{ND} pre-amphetamine) on calcitriol (blue) vs. placebo (grey) test days. **Figure 2B.** Calcitriol augmentation of amphetamine-induced improvements in vigilance/attention (DPRIME values) as measured by the Continuous Performance Task-AX (CPT-AX).

3. **Research Plan:** Summarize the study design and research procedures using non-technical language that can be readily understood by someone outside the discipline. **Be sure to distinguish between standard of care vs. research procedures when applicable, and include any flowcharts of visits specifying their individual times and lengths**. Describe the setting in which the research will take place.

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A total of 30 healthy subjects will be studied as outpatients as part of Specific Aims 1 & 2. All subjects will participate in total of four high-resolution PET scans using [^{11}C]-PHNO as the tracer (both baseline and post-Amp scans on both calcitriol and placebo pretreatment days). This is a within-subject, two-day four-scan, randomized, double-blind, placebo-controlled study design. In Aims 3 & 4, a total of 30 ADHD subjects will be studied as either inpatients or outpatients. They will complete same number of scans as healthy controls following same design.

In-Person Screening: Subjects who pass the telephone screening for this study will be invited to the CNRU for an in-person screening appointment, which will last approximately 2 hours. The purpose of this evaluation is to ensure that subjects meet study criteria (as listed below in section for inclusion/exclusion criteria). Subjects will be consented for the study, before the remaining screening procedures begin. The screening procedures include: a physical exam by a study physician, complete blood count (CBC) with differential, chemistry profile (including serum calcium and phosphate levels), thyroid function studies, LFTs, BUN, creatinine, serum 25(OH) vitamin D level, levels of parathyroid hormone, urine toxicology screening, urinalysis, cardiac screening questionnaire, and electrocardiogram (ECG). The amount of blood drawn during the screening visit is less than 60 milliliters (ml). All female subjects will undergo urine or serum pregnancy tests at the time of screening which must be negative in order to be considered for participation in the study. The total volume of blood collected throughout the duration of this study will be no more than 16 ounces which is well within the Red Cross blood donation standards and are safe for study participants. Subjects will also complete a structured clinical interview (SCID) (First 1995), a Magnetic Resonance Safety Screening Questionnaire, and a release of information so that study staff can contact the subject's primary care physician or can review medical records, if available and if considered necessary by the study physician. Once subjects are considered eligible, research pharmacy will randomly assign their first experimental day to calcitriol or placebo. The in-person screening visit will last approximately 2 hours. In-person screening will be the same for ADHD subjects with the addition of the Adult ADHD Self-Report Screening Scale for DSM-5 [6] as well as the ADHD component of the Structured Clinical Interview for DSM-5 (SCID) (First et al., 1995).

Magnetic Resonance Imaging (MRI): Magnetic resonance imaging (MRI) scans (3 T) will be collected in each subject for the purposes of excluding participants with anatomical abnormalities and anatomically co-registering PET and MRI for image analysis. A member of the research team will accompany each subject to the MRI Center and remain with the subject for the duration of the scan session. The acquisition sequence is a 3D MPRAGE MR pulse sequence with TE = 3.3 ms, flip angle = 7 degrees; slice thickness = 1.0 mm, 0.98 mm pixels. Personnel will escort the subject to the MRI Center. All female subjects will undergo a urine or serum pregnancy test before the MRI. The MRI scan will be completed on a separate day after the screening visit and before the experiment days (e.g., PET scans), and will last approximately 1 hour. Once subjects complete MRI, research staff will give them 3 capsules of the study medication (e.g., each capsule will have

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calcitriol 0.5 mcg or placebo). Subjects will receive instructions to keep these tablets until the night before the first experiment day and will also receive a sheet with instructions in order to prepare them for the first experiment day.

Night before the first experiment day: At 9 pm, the Principal Investigator (or other research staff member) will have a video (e.g., Skype or Facetime) conference call with subjects. The purpose of this conference call is to observe and confirm that subjects take the study medication. As part of this call: 1) research staff member will assess compliance with instructions to minimize risks from calcitriol (e.g., verify that subject had adhered to a diet low on calcium content that day and that they had drank 3-4 cups of water or electrolyte containing fluids above their usual fluid intake); 2) subjects will display the capsules; and 3) subjects will take the three capsules with one full glass of water. If participants are inpatient on the unit, medications will be administered at 9 pm by clinical nursing staff.

Test days: Each subject will be scheduled for two different visits at the Yale PET Center, separated by at least six days (based on the duration of calcitriol's pharmacological activity of 3-5 days) (Table 1). Each test day will start approximately at 8:00 am with verification that subjects were fasting (e.g., "NPO") since 11:59 pm the night before, followed by measurement of vital signs, an electrocardiogram, rapid dipstick urinary pregnancy test, and a blood draw of 10 milliliters (ml) to measure serum 1-25 $(OH)_2$ D_3 and calcium levels. Afterwards subjects will have oral administration of the study medication (e.g., three 0.5 mcg capsules of calcitriol vs. placebo). Thirty-five minutes after administration of the study medication, subjects will complete two different brief self-ratings: The Drug Effects Questionnaires and the Simplified Version of the Amphetamine Interview Rating Scale (Van Kammen and Murphy 1975, Morean, de Wit et al. 2013). Before the baseline scan, subjects will complete the eye blink rate and the Probabilistic Reversal Learning Task (PRLT).

Preparation of the subject for PET scanning will include the placement of a venous line. The purpose of the venous line is for the [^{11}C]-PHNO injection; the venous line may also be used for blood sampling. Approximately two hours (i.e., 120 minutes) after calcitriol administration, subjects will have IV administration of a dose of up to 10 mCi of [^{11}C]-PHNO. Then each subject will undergo a 120-minute dynamic (i.e., emission and transmission) scan on a high-resolution research tomography (HRRT). Ninety minutes after starting the [^{11}C]-PHNO PET scan, subjects will have blood drawn for measurement of calcitriol levels. Upon completion of the [^{11}C]-PHNO PET scan, subjects will complete a 20-minute, computer-based task of attention, the CPT-IP (i.e. continuous performance task). Approximately 4.5 hours after receiving the study medication, subjects will receive an oral dose of dextro-amphetamine (Dexedrine 0.3 mg/kg, to a maximum dose of 30 mg). Subjects will have blood drawn for the measurement of amphetamine levels at 60 and 120 minutes after receiving dextro-amphetamine. Before the second scan, subjects will complete the eye blink rate. Approximately two and a half hours after receiving dextro-

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amphetamine, subjects will have a second IV administration of up to 10 mCi of [^{11}C]-PHNO, followed by another 120-minute dynamic (i.e., emission and transmission) imaging session scan on a HRRT. Ninety minutes after starting the second [^{11}C]-PHNO scan, subjects will have blood drawn for the measurement of calcitriol, calcium (Ca), and Phosphorus (P) levels. Upon completion of the second [^{11}C]-PHNO scan, subjects will complete the 20-minute CPT-IP a second time. A summary of procedures described above is provided in Table 1. End of the test day will be after subject has tolerated lunch and has been cleared by the study physician for discharge. The amount of blood drawn during each experiment day is less than 70 ml.

If participating as an outpatient, ADHD subjects study will follow the exact same study design and follow all of the same study procedures as healthy controls, but will take their prescribed stimulant medication, at their normal prescribed dose, in place of the dextro-amphetamine. These subjects will not be asked to discontinue their medication leading up to or throughout the study procedures.

If participating as an inpatient, ADHD subjects will follow the same MRI procedures, study procedures night before each experiment day, and test day procedures, with the main variation being that they will be admitted to the CNRU while they complete those. The CNRU is an inpatient, research dedicated, research unit, staffed 24 X 7 with medical and nursing staff, where visitors are screening/restricted. Upon admission to CNRU, subjects will conduct admission procedures including medical and psychiatric examination, blood work for CBC with differential, comprehensive metabolic panel with TSH and serum pregnancy test, urinalysis, ECG, and urine toxicology. The total length of stay on the CNRU will be up to 3 weeks depending on days needed to complete 5 half lives off stimulant medications and depending on slots for PET imaging scan which will be schedule at least 6 days apart from each other. The first days on the CNRU are meant to acclimatized to the inpatient environment. Congruent with inpatient study procedures: they will not need to receive study medication to take home after MRI; they don't need to have administration of study medication monitored by Skype/Facetime; compliance with dietary measurments before test days is supervised by staff from CNRU; and they will be returning from the PET Imaging Center back to the CNRU after completion of each test day.

Follow up: The day after completion of each test day, subjects will be asked to return to the CNRU for a blood draw in order to measure calcium levels. The amount of blood drawn will be less than 5 ml. The purpose of this procedure is to monitor the development of hypercalcemia as an adverse event. In addition, a study physician will interview subjects in order to assess any potential residual side effects relative to their pre-scan baseline. At the end of the first follow up visit, subjects will receive 3 capsules of the study medication (i.e., each capsule will have calcitriol 0.5 mcg or placebo) as well as another instruction sheet for the second experimental day. The second experimental day is the same as the first one.

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ADHD outpatient subjects will complete the same follow up. Inpatient subjects, because they are residing on the CNRU during those days, don't need to receive either the study medication or instructions for the next experiment day, as part of their follow up.

Dosing: We will use a threshold Dexedrine dose of 0.3 mg/kg (as oppose to 0.4 mg/kg or 0.5 mg/kg in order to minimize the potential for ceiling effects on DA release/[^{11}C]-PHNO displacement, thereby maximizing the chances of detecting calcitriol related augmentation of stimulant-induced DA release.

The choice of calcitriol dose (3 mcg) is based on previous studies in both rodents and humans (Broadus, Erickson et al. 1984, Erickson, Cooper et al. 1984, Bianchi, Ardissino et al. 1999, Brandi, Egfjord et al. 2002, Beer, Eilers et al. 2003, Beer, Ryan et al. 2007, Cass, Peters et al. 2012). Such studies have employed higher doses than physiologic ones (i.e., those employed in the treatment of vitamin D deficiency) and have demonstrated the safety of oral doses as high as 0.5 mcg/kg (among persons with malignancies) (Beer, Lemmon et al. 2003) or oral/IV doses as high as 4 mcg (among healthy controls) (Brandi, Egfjord et al. 2002). Studies in healthy controls (N=11) using a protocol of 3 mcg of daily oral calcitriol for 10 consecutive days (Broadus, Erickson et al. 1984) have been shown to be safe and well-tolerated, with a single subject developing mild hypercalcemia after six days (this subjects continued in the study at a reduced dose of 1.5 mcg per day without complications). In the current study, the total dose of 3 mcg will be divided in two separate administrations of 1.5 mcg for ease of administration (e.g., three capsules will be easier than six), to maximize chances that [^{11}C]-PHNO scans are conducted after calcitriol has had sufficient time to take effect (e.g., onset of action after 2 hours; maximum effect after 10 hours; duration of effect is 3 to 5 days), and to maximize chances of subjects tolerating it/minimizing chances of any adverse events.

Doses of calcitriol and Dexedrine (if applicable) administered to ADHD subjects are the same ones as the ones administered to healthy controls. ADHD outpatient subjects will take the normal, prescribed dose of their prescription stimulant medication.

Table 1. Timeline of Study Procedures ^

Study Procedure	Night Before Day 1	Day 1	Night Before Day 2	Day 2	Time Relative to Scan #1	Time Relative to Scan #2
Pretreatment Dose 1 (Placebo vs. Calcitriol)	✓		✓			- 18 hr
Baseline Vital Signs		✓		✓	- 130 min	
ECG and Blood Draw (Calcitriol and Ca^{2+})		✓		✓	- 125 min	
Pretreatment Dose 2 (Placebo vs. Calcitriol)		✓		✓	- 120 min	- 7 hr
Self-Ratings (DEQ and AIRS*)		✓		✓	- 90 min (and Q45 min thereafter)	

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Vital Signs		✓		✓	- 75 min (Q 15 min thereafter until stable after end of Scan #2)	
Eye blink rate		✓		✓	-60 min	
PRLT**		✓		✓	-45 min	
Cardiac Monitoring (Rate & Rhythm)		✓		✓	- 15 min (and continuously thereafter until stable after Scan #2)	
[^{11}C]-PHNO injection & PET Scan #1		✓		✓	0	- 5 hr
Blood Draw (Calcitriol)		✓		✓	+ 90 min	
CPT-IP***		✓		✓	+ 120 min	
Dexedrine Dose (0.3 mg/kg PO) or Prescription Stimulant		✓		✓		- 150 min
Blood Draw (Amphetamine)		✓		✓		- 90 min
Blood Draw (Amphetamine)		✓		✓		- 30 min
Eye blink rate		✓		✓		- 15 min
[^{11}C]-PHNO injection & PET Scan #2		✓		✓		0
Blood Draw (Calcitriol, Ca, and P)		✓		✓		+ 90 min
CPT-IP***		✓		✓		+ 120 min

*Drug Effects Questionnaire and simplified Amphetamine Interview Rating Scale
**Probabilistic Reversal Learning Task
***Continuous Performance Task – IP
^ Table applies to both healthy controls and ADHD subjects

Vitamin D measurements: In the context of vitamin D deficiency, 1-25$(OH)_2D_3$ (calcitriol) serum level measurement has insignificant diagnostic value. Instead 25$(OH)D_3$ level (not the active form) is widely measured as a marker of body vitamin D status (Holick 2009). In our study we will measure 25$(OH)D_3$ serum levels once, as part of the screening procedures (below 12 ng/ml will be set as the threshold to diagnose vitamin D deficiency/exclude subjects from the study). We will use the IDS-iSYS autoanalyzer from Immunodiagnostic Systems, for the measurement of 25$(OH)D_3$. This assay is based on chemiluminescence technology (Boscato and Stuart 1988, Sempos, Vesper et al. 2012, Thienpont, Stepman et al. 2012).

Once the screening is completed, our main focus will be 1-25$(OH)_2D_3$ measurements on each test day since we are pre-treating our subjects with calcitriol. There is conflicting evidence regarding the relationship between these two values worldwide (Need, Horowitz et al. 2000, Need, O'Loughlin et al. 2008). Serum calcitriol levels will be measured via IDS-iSYS 1,25-Dihydroxy Vitamin D kit (Hussein, Ibrahim et al. 2015) three times on each experimental day, once prior to calcitriol/placebo administration, and within 90 minutes of each [^{11}C]-PHNO scan.

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PET imaging: PET scans are acquired as subjects rest on a high-resolution research tomography HRRT (207 slices, with a 1.2 mm slice separation and a reconstructed image resolution of ~3 mm). This resolution permits visualization of [^{11}C]-PHNO uptake in the caudate, putamen, ventral striatum (VST), and substantia nigra / ventral tegmental area (SN/VTA). Partial immobilization of the head will be done using Velcro strips and Coban. In addition, on the HRRT scanner, a sensor will be attached to the Coban on the subject's head which will allow for tracking and correction of any head movement during the scan. An attenuation correction scan is obtained immediately before or after each emission scan.

The PET scans will be acquired using i.v. bolus administration of 10 mCi dose of high-specific activity [^{11}C]-PHNO (maximum mass dose of 0.03 μg/kg) as described by Gallezot and colleagues (Gallezot, Beaver et al. 2012). Each dynamic PET scan will last up to 120 minutes (including attenuation correction and emmission scan). Subjects will be asked to void immediately after the scan is completed, to reduce radiation exposure to the bladder. Each experimental day may last from 8 am to 5 pm.

The primary outcome of DA displacement will be post-Amp BP_{ND} in comparison to baseline BP_{ND}. We will conduct baseline scans on both days in order to rule out potential effects of calcitriol on D2/3 BP_{ND}.

ADHD subjects will complete same PET imaging study procedures as healthy controls.

Continuous Performance Task (CPT-IP): The Continuous Performance Task is one the most widely used tests to quantify attention deficits and attentional changes induced by pharmacological agents in both clinical and research settings. It is a modified version of the older CPT-AX, giving it higher sensitivity to detect more subtle changes as well as also measuring working memory (Rapisarda et al. 2014) (Cornblatt et al. 1988). In this computer based test the subjects are shown a random sequence of different numbers and are instructed to press a button as quickly and accurately as possible (with their preferred hand) upon detection of two identical pairs of numbers, and to withhold their response to any other sequence of letters. In order to ensure appropriate completion of the task, subjects will complete the task while video conferencing with research team using zoom, allowing virtual sharing of the screen.

ADHD subjects will also complete CPT-IP.

Probabilistic Reversal Learning Task (PRLT): The computer based PRLT (Swainson, 2000) measures subjects' perseverative responding in the context of changing reward contingencies / cues.

ADHD subjects will also complete PRLT.

Eyeblink Rate: Preclinical research in non-human primates (Taylor et al. 1999) and clinical

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studies of humans (e.g., Parkinson's disease) have shown eyeblink rate to be influenced by subcortical DA (with blink rate positively correlated with endogenous DA levels)(98). Thus, we will *explore* the utility of eyeblink as a behavioral proxy ("poor man's PET scan") for assessing calcitriol-induced changes in basal and/or amphetamine-stimulated DA release. If sensitive to calcitriol effects, such methods might provide an efficient and cost-effect technique for future investigations. Eyeblink rate will be measured before all PET scans. In this optional task, participants will be asked to stare into a camera and remain still yet act relaxed for a period of five minutes. This will be recorded and saved as a video that is not associated with the name of the participant. A script will be used through Anaconda Python Distribution to identify and code when the participant blinks during the recorded period. This task aims to assess frequency and variability of eye blinks between minute long periods to examine a potential relation to impulsive based behaviors.

ADHD subjects will also have the option to complete Eyeblink Rate.

Drug Effects Questionnaire (DEQ): The Drug Effects Questionnaire (DEQ) is used in studies of subjective response (SR) to a variety of substances, such as amphetamines. It assesses the extent to which participants feel any substance effect, feel high, like the effects, dislike the effects, and want more of the substance. These five constructs are summarized as FEEL, HIGH, DISLIKE, LIKE, and MORE (Hamilton, Strader et al. 2011).

ADHD subjects will also complete DEQ questionnaires.

Simplified version of the amphetamine interview rating scale: Behavioral response to amphetamine will be measured by self-ratings with a simplified version of the Amphetamine Interview Rating Scale (Van Kammen and Murphy 1975). Four items will be investigated: euphoria ("feel good"), alertness ("feel energetic"), restlessness ("feel like moving") and anxiety ("feel anxious").

ADHD subjects will also complete simplified version of the amphetamine interview rating scale.

Structured Clinical Interview for DSM-V (SCID): The SCID is a semi-structured, clinician-administered interview for making major DSM-V diagnoses (First et al., 1995). It will be used in healthy controls to confirm absence of exclusion criteria. It will be used in ADHD subjects to confirme diagnosis of ADHD as well as to confirm absence of exclusion criteria.

4. Genetic Testing N/A X

A. Describe

i. the types of future research to be conducted using the materials, specifying if immortalization of cell lines, whole exome or genome sequencing, genome wide association studies, or animal studies are planned

ii. the plan for the collection of material or the conditions under which material will be received

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iii. the types of information about the donor/individual contributors that will be entered into a database
iv. the methods to uphold confidentiality

B. What are the conditions or procedures for sharing of materials and/or distributing for future research projects?
C. Is widespread sharing of materials planned?
D. When and under what conditions will materials be stripped of all identifiers?
E. Can donor-subjects withdraw their materials at any time, and/or withdraw the identifiers that connect them to their materials?
    i. How will requests to withdraw materials be handled (e.g., material no longer identified: that is, anonymized) or material destroyed)?

F. Describe the provisions for protection of participant privacy
G. Describe the methods for the security of storage and sharing of materials

5. Subject Population: Provide a detailed description of the types of human subjects who will be recruited into this study.

We will recruit 30 healthy subjects and 30 ADHD subjects, both males and females between the ages of 18-50 years old. These subjects will be medically/psychologically healthy (as described below). ADHD subjects will also be medically/psychologically healthy. Enrolling 30 healthy subjects and 30 subjects with ADHD in the study will allow us to account for subject loss to follow-up and technical issues during data collection that can preclude the interpretation of data.

6. Subject classification: Check off all classifications of subjects that will be <u>specifically recruited for enrollment</u> in the research project. Will subjects who may require additional safeguards or other considerations be enrolled in the study? If so, identify the population of subjects requiring special safeguards and provide a justification for their involvement.

☐ Children	☒ Healthy	☐ Fetal material, placenta, or dead fetus
☐ Non-English Speaking	☐ Prisoners	☐ Economically disadvantaged persons
☐ Decisionally Impaired	☐ Employees	☐ Pregnant women and/or fetuses
☐ Yale Students	☐ Females of childbearing potential	

NOTE: Is this research proposal designed to enroll children who are wards of the state as potential subjects? ☐ Yes ☒ No (If yes, see Instructions section VII #4 for further requirements)

7. Inclusion/Exclusion Criteria: What are the criteria used to determine subject inclusion or exclusion?

<u>Inclusion Criteria:</u>
1) Age 18-50 years

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2) Voluntary, written, informed consent
3) Physically healthy by medical history, physical, neurological, ECG, and laboratory examinations
4) For females, non-lactating, with a negative serum or urine pregnancy (β-HCG) test
5) Lab results without clinically relevant findings (e.g. renal function, electrolytes, and vitamin D levels)
6) English speaking

Exclusion criteria:

1) Medical contraindication to Dexedrine administration (e.g., history of cardiac problems, seizures, glaucoma, hypertension, hyperthyroidism, etc.)
2) Medical contraindication to calcitriol administration (e.g., history of hypersensitivity to calcitriol or any component of the formulation, hypercalcemia or vitamin D toxicity)
3) History of substance dependence (e.g., alcohol, opiates, sedative hypnotics), except for nicotine (ADHD participants could be dependent on stimulants)
4) A primary major DSM-V psychiatric disorder (e.g., schizophrenia, bipolar disorder, major depression, etc.) as determined by the Structured Clinical Interview for DSM-V (SCID)
5) A history of significant medical (e.g., cardiovascular, diabetic/metabolic) or neurological (e.g., cerebrovascular accidents, seizure, traumatic brain injury) illness
6) Positive answers on the cardiac history questionnaire that may place the subject at higher risk, as determined by an internal medicine specialist or cardiologist's review of both the questionnaire responses and screening ECG
7) Current use of psychotropic and/or potentially psychoactive prescription medications
8) For females, laboratory (β-HCG) or physical evidence of pregnancy/lactation
9) MRI-incompatible implants and other contraindications for MRI (i.e., aneurysm clip, metal fragments, internal electrical devices such as a cochlear implant, spinal cord stimulator or pacemaker)
10) History of claustrophobia or feeling of inability to lie still on his/her back for the PET or MRI scans
11) History of any bleeding disorder or current anticoagulant therapy
12) Donation or loss of 550 mL of blood or more (including plasmapheresis) or receipt of a transfusion of any blood product within 8 weeks prior to the first test day.
13) Use of any prescription medications and/or over-the-counter medications, vitamins and/or herbal supplements which could have a negative clinical interaction with calcitriol/Dexedrine or which could confound scientific results of the study, within 2 weeks prior to each test day (e.g., thiazide diuretics, Mg based antiacids, digoxin, etc,).
14) Serum levels of $25(OH)D_3$ below 12 ng/ml.
15) Morbid obesity i.e. BMI over 35 (more prone to lower vitamin D levels)

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16) Subjects with history of prior radiation exposure for research purposes within the past year such that participation in this study would place them over FDA limits for annual radiation exposure. This guideline is an effective dose of 5 rem received per year.
17) Subjects with current, past or anticipated exposure to radiation in the work place
18) History of kidney stones within the past 5 years
19) Any degree of renal failure
20) History of parathyroid disorder (hyper or hypoparathyroidism)
21) History of osteoporosis or any pathologic fractures
22) Vitamin D supplementation in any form in the past 3 months
23) Known hypersensitivity to Dexedrine, [^{11}C]-PHNO, or calcitriol
24) Malabsorption syndromes (i.e. Celiac sprue)
25) Serum corrected calcium > 10.5 mg/dl or phosphate > 4.2 mg/dl

ADHD subjects will have the same inclusion/exclusion criteria as healthy controls with the following exceptions: They have DSM-5 diagnosis of ADHD (including individuals with a childhood/adult history of stimulant exposure and/or current stimulant use based on verbal report and/or positive serum/urine drug levels). Inpatient subjects will be stimulant-free at the time of PET scanning (>5 half-lives off stimulants). Outpatient subjects must have a verified prescription for stimulant medication and will continue to take it throughout the study as prescribed.

8. How will **eligibility** be determined, and by whom?

Physical health will be determined via medical history, a physical examination, neurological, ECG and laboratory examinations. DSM-V criteria for absence of primary Axis I psychiatric disorder and history of other substance dependence will be determined by the Structured Clinical Interview for DSM-V (SCID) performed by a member of the research staff. In addition, medical or psychiatric information can be gathered as part of the screening by reviewing medical records or by talking with primary care providers. For outpatient ADHD subjects, a release of information is necessary so that study staff can contact their primary care physician or prescriber of stimulants, and can have access to the Connecticut Prescription Monitoring Program (CTPMP) in order to confirm that the subject is being prescribed stimulants (in addition to visible evidence of ongoing prescription use by virtue of a current active pharmacy dispensed prescription bottle). Contraindications to participate in PET imaging procedures will be assessed by looking at the Yale PET Center Database, which has information about prior participation in research studies. Contraindications to participate in the MRI will be assessed with the MR safety questionnaire. All female subjects will undergo serum or urine pregnancy (β-HCG) test to determine evidence of pregnancy. Once screening is completed, research assistant will meet with study physician and will go over eligibility checklist. Eligibility for ADHD subjects will follow the same study procedures with the addition of the Adult ADHD Self-Report Screening Scale for DSM-5 as well

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as the SCID section for ADHD.

9. Risks: Describe the reasonably foreseeable risks, including risks to subject privacy, discomforts, or inconveniences associated with subjects participating in the research.

The risks from this study include 1) Risks associated with unknowns, 2) Risks associated with intravenous line placement and blood drawing, 3) Risks associated with PET scans, 4) Risks associated with MRI scans, 5) Risks associated with calcitriol administration, 6) Risks associated with amphetamine (e.g., Dexedrine) administration, 7) Risks associated with questionnaires and interviews, 8) Risks associated with neurocognitive testing, and 9) Privacy/loss of confidentiality.

9.1. Risks Associated with Unknowns: The subject's health and safety will always be the primary concern of the doctors and staff performing the study. In the event of an unexpected outcome, all necessary medical action will be taken. Medication might be administered as needed, per the Yale PET Center standard operating procedure for medical emergencies, in order to treat complications.

9.2. Phlebotomy and intravenous line: As part of the screening assessments of medical eligibility and the [^{11}C]-PHNO infusion during the test days, subjects will undergo phlebotomy and intravenous cannulation, respectively. Drawing blood and inserting an intravenous line (IV) into an arm vein are safe and standard medical procedures. Venous sampling may be associated with mild-to- moderate pain or bruising at the puncture site. Bruising and thrombosis can occur during phlebotomy and the placement of the intravenous line. In rare instances poor healing, or infection at the catheter insertion site may occur. Certain individuals may feel light-headed during venipuncture. The volume of blood collected during this study, may include screening laboratories, MRI and PET scans, will be less than 15 tablespoons.

9.3. Risks associated with the PET scans/ radiation exposure: The Yale University Radioactive Drug Research Committee (YNHH RSC) will review the use of radiation in this research study and no subjects will be scanned until RSC approval is obtained. This research study involves exposure to radiation from PET scanning. This radiation exposure is not necessary for medical care and is for research purposes only. The targeted amount of radiation an individual subject will receive in this study is from four injections of [^{11}C]-PHNO ($\leq$10 mCi each injection) plus associated transmission scans. However, in situations where a PET scan is not successful following a PET injection (e.g., problems with the PET camera) the subject may receive an additional PET injection, up to a total of five injections during the study, if deemed appropriate and below the maximum amount of radiation exposure allowed. Although each organ will receive a different dose, the amount of radiation received from each 10mCi injection is approximately 0.167 rem. The maximum amount of radiation exposure one may receive from this study is approximately 0.847 rem (taking into account a maximum of 5 possible 10mCi

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[^{11}C]-PHNO PET tracer injections should an issue arise with the PET camera). This amount of radiation exposure is below the annual limit of 5 rem set by the FDA and monitored by the Yale New Haven Hospital Radioactive Drug Research Committee (Y-NHH RSC) for research subjects.

The effects of radiation exposure on humans have been studied for over 60 years and no harmful effect to humans has been observed from the levels of radiation subjects will receive while taking part in this research study. However, scientists disagree on whether radiation doses at these levels are harmful. It is unclear whether already low doses of radiation could cause cancer. Even though no effects have been observed, some scientists believe that radiation can be harmful at any dose - even low doses such as those received during this research.

Another concern some people may have about radiation exposure is the effect on fertility or on the possibility of causing harm to future children (i.e., genetic effects). The doses subjects will receive in the study are well below the levels needed to affect fertility. In addition, genetic effects have not been seen in humans who have been exposed to radiation. The information on genetic effects currently available is based on animal studies using much larger doses of radiation than the amount individuals will receive in this study.

Additional discomfort associated with receiving [^{11}C]-PHNO:

[^{11}C]-PHNO has been used in humans and has been shown to be *safe and well tolerated after its administration to healthy subjects and patients.* At the Yale PET Center, over 250 administrations of [^{11}C]-PHNO have already occurred. One unexpected adverse effect (unresponsiveness for approximately one minute 12 minutes post-injection of [^{11}C]-PHNO) was reported and was likely due to underlying cardiac history that was possibly exacerbated by study procedures. A comprehensive review of vital signs data was conducted on all subjects injected with [^{11}C]-PHNO at the Yale University PET Center and showed no correlation or relationship of any kind between injection of [^{11}C]-PHNO and systolic blood pressure, diastolic blood pressure, or heart rate measures.

The most common adverse event reported has been self-limiting transient nausea shortly after injection (well before the peak brain uptake). The injected mass dose is limited to 0.03 μg/kg to minimize this effect. Several human subjects who have participated in PET studies using [^{11}C]-PHNO in Toronto, Canada, have experienced nausea, emesis and other effects such as dizziness, headache or a warm sensation. Although the occurrence of these side effects had no relation with the injected mass, subject's sex, body mass or age, the mechanism of these reactions is not understood, but it represents a potential risk. Nausea has been attributed to the transient high [^{11}C]-PHNO concentration 2–3 min after the bolus injection of the tracer (Willeit, Ginovart et al. 2006).

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Other side effects, such as nausea/vomiting, dizziness, headache, epigastric pain, insomnia, psychosis, cardiac arrhythmia, hypertention, and psychosis have been reported when [^{11}C]-PHNO is administered with amphetamines. These effects were not present during the timeframe between [^{11}C]-PHNO and amphetamine but after the amphetamine administration.

9.4. Risks associated with MRI scans: MR carries a risk for subjects who are claustrophobia or have pacemakers, metal pieces, aneurysm clips, large colored tattoos, or any other contraindications for MR. Magnetic resonance (MR) is a technique that uses magnetism and radio waves, not x-rays, to take pictures and measure chemicals of various parts of the body. The United States Food and Drug Administration (FDA) has set guidelines for magnet strength and exposure to radio waves, and we carefully observe those guidelines. Subjects will be watched closely throughout the MR study. Some people may feel uncomfortable or anxious. If this happens, the subject may ask to stop the study at any time and we will take them out of the MR scanner. On rare occasions, some people might feel dizzy, get an upset stomach, have a metallic taste or feel tingling sensations or muscle twitches. These sensations usually go away quickly but we will ask subjects to tell the research staff if they have them. There are some risks with an MR study for certain people. If subjects have a pacemaker or some metal objects inside their body, they may not be in this study because the strong magnets in the MR scanner might harm them. Another risk is the possibility of metal objects being pulled into the magnet and hitting a subject. This MR study is for research purposes only and is not in any way a clinical examination. The scans performed in this study are not designed to find abnormalities. The primary investigator, the lab, the MR technologist, and the Magnetic Resonance Research Center are not qualified to interpret the MR scans and are not responsible for providing a diagnostic evaluation of the images. If a worrisome finding is seen on a subject's scan, a radiologist or another physician will be asked to review the relevant images. Based on his or her recommendation (if any), the primary investigator or consulting physician will contact the subject, inform them of the finding, and recommend that they seek medical advice as a precautionary measure. The decision for additional examination or treatment would lie solely with the subject and their physician. The investigators, the consulting physician, the Magnetic Resonance Research Center, and Yale University are not responsible for any examination or treatment that a subject receives based on these findings. The images collected in this study are not a clinical MR exam and for that reason, they will not be made available for diagnostic purposes.

9.5. Risks associated with calcitriol administration: Potential side effects of calcitriol are cardiac arrhythmia, hypertension, apathy, drowsiness, headache, hyperthermia, metallic taste, psychosis, sensory disturbance, erythema, erythema multiforme, pruritus, skin rash, urticaria, albuminuria, calcinosis, decreased libido, dehydration, growth suppression, hypercalcemia, hypercholesterolemia, polydipsia, weight loss, abdominal pain, anorexia, constipation, nausea, pancreatitis, stomach pain, vomiting, xerostomia, hypercalciuria, nocturia, urinary tract infection, increased serum ALT, increased serum AST, hypersensitivity reaction, dystrophy, myalgia,

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ostealgia, weakness, conjunctivitis, photophobia, calcium nephrolithiasis, increased blood urea nitrogen, increased serum creatinine, polyuria, and rhinorrhea.

Although calcitriol doses of 3 mcg are higher than doses used in treatment of vitamin D deficiency in kidney failure patients, they have been safely used in prior studies. In one study using higher doses of calcitriol than current study (e.g., up to 45 micrograms or 0.6 mcg/kg for a person whose weight is 70 kg, once per week, for three consecutive weeks), only one case of symptomatic kidney stone was reported among 125 patients (Beer, Ryan et al. 2007). It is unlikely (but yet possible) to have hypercalcemia in our study. Phase I studies weekly administration (e.g., up to 4 weeks) of doses higher than 0.48 mcg/kg showed self-limiting grade 1 hypercalcemia (Beer, Munar et al. 2001) (Beer, Ryan et al. 2007) which resolved without treatment. Studies with healthy controls (N = 11) using doses of 3 mcg per day (equivalent to the maximum dose allowed in this study), for 10 consecutive days, revealed one case of mild hypercalcemia on day 6. This subject continued the study at doses of 1.5 mcg per day (Broadus, Erickson et al. 1984). Other studies with healthy controls who don't have any malignancies administered 4 mcg of calcitriol intravenously or 1.5 mcg mcg/m^2 without significant adverse events (Bianchi, Ardissino et al. 1999, Brandi, Egfjord et al. 2002).

9.6. Risks associated with amphetamine administration: Risks of amphetamine administration include both medical and psychiatric risks. Potential side effects of d-amphetamine administration are cardiomyopathy, hypertension, palpitations, tachycardia, aggressive behavior, dizziness, dysphoria, euphoria, exacerbation of tics, gilles de la Tourette's syndrome, headache, insomnia, mania, overstimulation, psychosis, restlessness, urticarial, change in libido, weight loss, anorexia, constipation, diarrhea, unpleasant taste, xerostomia, frequent erections, impotence, prolonged erection, dyskinesia, rhabdomyolysis, tremor, accommodation disturbances, and blurred vision. The frequency for several of the above side effects is not well defined. The frequent somatic side effects of d-amphetamine administration are cardiovascular (e.g. hypertension, palpitations, and tachycardia). General effects such as sweating, feeling warm or cold, nausea, diarrhea, muscle and abdominal cramping, have been reported frequently. Behavioral effects in this dose range are increased level of alertness, talkativeness, restlessness, agitation, mood changes (usually euphoria), and anxiety. In 2015, warnings on the risk of suicidal thoughts and behaviors were being incorporated into the Canadian prescribing information for drugs used in the management of attention-deficit/hyperactivity disorder (ADHD). The new warnings advised that there have been reports of suicide-related events including thoughts of suicide, suicide attempts, and completed suicide.

In our experience, effects such as increased level of alertness, talkativeness, restlessness, agitation, and mood changes are generally transient and well tolerated. This dose of amphetamine has not been reported to induce psychotic symptoms in non-schizophrenic subjects. Infrequently blurred vision, headaches and chest tightness, and changes in EKG have been reported. There is a rare risk of permanent neurological damage and death as a result of cardiac

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arrest or stroke.

9.7. Risks associated with questionnaires and interviews: There are no known significant risks associated with the completion of interviews/questionnaires. However, subjects may become tired and may feel uncomfortable answering some questions about psychiatric or substance use disorders.

9.8. Risks associated with neurocognitive testing: There are not specific risks associated with this. However; subjects may experience some frustration with some tests if they find them difficult or if they perceive that their performance is not as good as they expected. They may also experience boredom if the test becomes repetitive. Given the attention these tasks could require, they may feel tired after completing them.

9.9. Privacy/loss of confidentiality: Since some of the data collected (e.g., history of substance use behavior) is private in nature and may have legal implications, risks related to loss of confidentiality also exist.

Risks will be the same for subjects with ADHD. Because of their history of exposure to stimulants, they are more likely to be tolerant/protected from risks associated with administration of amphetamines.

10. Minimizing Risks: Describe the manner in which the above-mentioned risks will be minimized.

10.1. Risks associated with Unknowns: In the event of an unexpected outcome, all necessary medical action will be taken. Medication might be administered as needed, per the Yale PET Center standard operating procedure for medical emergencies, in order to treat complications.

10.2. Risks of phlebotomy and intravenous line: The risks of bruising, clotting, and infection will be minimized by having these procedures performed by experienced personnel using aseptic technique and by exclusion of subjects with medical or laboratory history compatible with bleeding disorders. Subjects will have less than 7.5 oz. of blood (e.g., equivalent to less than 15 table spoons) drawn throughout the duration of the study. These amounts are well within the Red Cross blood standards. To avoid injury due to fainting, the catheter will be inserted when the subjects are in a seated/recumbent position. The blood draws will be obtained from the already inserted cannula, to minimize discomfort. Infection is avoided by adequate cleansing of the skin, prior to intravascular line insertion, and by the exclusion of immune-compromised subjects. When the IV catheter is removed, any bleeding will be stopped and the site will be covered with a clean dressing. Subjects who have donated blood within 8 weeks of the present study will be excluded. Participants will be told that they should not give blood for at least 8 weeks.

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10.3. Risks of PET Procedures: This dose of radiation will be submitted for approval to the Yale New Haven Hospital Radiation Safety Committee (Y-NHH RSC). All scans will be done in the presence of medical supervision and trained nursing staff in an institution specifically designed to support imaging studies. In the event of serious medical complications, the Yale University PET scan facilities have immediate access to or consultation with specialized medical units at the Yale-New Haven Hospital. Preparation of radiopharmaceuticals and execution of PET scans will be performed by radiochemists, physicians, and technologists of the Department of Diagnostic Radiology, Yale University School of Medicine. These professionals are qualified by training and experience in the safe use and handling of radionuclides. Subjects will be asked about their previous radiation exposure, and those who have had research exposure within the past year will be excluded if their cumulative annual exposure (including the present study) exceeds FDA limits. Examples of the types of radiation exposure considered include x-rays taken in radiology departments, cardiac catheterization, and fluoroscopy as well as nuclear medicine scans in which radioactive materials were injected into their body.

No PET studies will be performed on pregnant or potentially pregnant women, as confirmed by pregnancy testing during evaluation and on each scan day before initiation of any scan procedures. If subjects are breastfeeding they will not be able to participate in this research study.

In terms of the additional risks associated with [^{11}C]-(+)-PHNO infusion, the most common adverse event reported has been self-limiting transient nausea shortly after injection (well before the peak brain uptake). Although the occurrence of these side effects had no relation with the injected mass, subject's sex, body mass or age, the mechanism of these reactions is not understood, but it represents a potential risk. Because nausea is attributed to the transient high [^{11}C]-PHNO concentration 2–3 min after the bolus injection of the tracer (Willeit, Ginovart et al. 2006), the Yale PET Center administers [^{11}C]-PHNO as a bolus over a period of 5 minutes, *i.e.*, at a PHNO dose rate of 7 ng/min for the average 70 kg subject. Administration of [^{11}C]PHNO dose at this rate is about 5 times slower than lowest rate reported in the clinical study involving i.v. administration of PHNO (Coleman, Quinn et al. 1990). Every effort will be taken to minimize these side effects and their impact on subjects participating in the study. In terms of risks related with combination of [^{11}C]-PHNO and amphetamines, we will be using doses of amphetamine (0.3 mg/kg) lower than the doses used in other studies (Up to 0.5 mg/kg).

10.4. Risks associated with the MRI scans: To minimize risks, each subject will fill out the Yale Magnetic Resonance Research Center MRI Safety Questionnaire before the study. Only subjects who fulfill the criteria by this questionnaire will be eligible for the study. A member of the research staff will accompany the subject to the scan console room or magnet room and stay there during the scanning. Subjects will remove all metal (watch, hair pins, jewelry) and walk through the metal detector in the Magnetic Resonance Research Center (MRRC) before entering the MRI room. If the subject has any metallic prostheses/implants they will be excluded from the study. If a subject becomes anxious during the scan they can request that the MRI scan be stopped.

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10.5. Risks of calcitriol administration: Subjects will be asked to withhold any supplemental calcium intake, as well as to decrease consumption of foods rich in calcium content (e.g. dairy products, foods with milk or milk products as a main ingredient, high-calcium vegetables, legumes, and seafood starting 24 hours prior to each test day until 48 hours after. They will be asked to drink 3-4 cups (24-32 ounces) of water or electrolyte containing fluids above their usual intake starting 12 hours before the dose. This oral hydration will be recommended for 3 days after dosing (Beer, Munar et al. 2001, Beer, Javle et al. 2007). Based on literature reviewed, it is not expected for subjects to have hypercalcemia but if they develop it, it is expected for it to by asymptomatic and to resolve spontaneously without the need for treatment. Notwithstanding the above, subjects will be educated about these symptoms (i.e. polyuria, polydipsia, nausea, vomiting, constipation). Serum calcium levels will be measured each experiment day, as well as the day after each experiment day, in order to detect asymptomatic cases of mild hypercalcemia. The study will be discontinued if the calcium levels are congruent with mild hypercalcemia or higher than 11.5 mg/dL. In these cases we will arrange for consultation with internal medicine specialists.

10.6. Risks associated with amphetamine administration: Subjects will be excluded if they have significant medical history (e.g., history of coronary artery disease, arrhythmias, etc,.), findings of physical examination (e.g., heart murmurs, uncontrolled high blood pressure, etc,.), abnormal routine blood tests (e.g., elevated BUN or creatinine, etc,.), urine toxicology (e.g., positive for cocaine, amphetamines, etc,.) and EKG (e.g., arrhythmia, etc,.) which could pose a risk in the context of dexedrine's administration. The study will be canceled on the day of the scan if several of the subject's blood pressure readings are recorded at >90 for diastolic BP or >140 for systolic BP while at rest. Any automated blood pressure results that are abnormal will be repeated manually. The manual reading will be the official reading. Inclusion in the study will be limited to individuals who are between the ages of 18-50. Patients will also be excluded if they have any h/o severe medical or neurological illness, any clinically significant brain abnormality, and if they have recently donated blood. Administration of oral d-amphetamine will take place at the PET center with a physician on site and with ACLS trained nursing staff. Frequent EKG and vital signs' monitoring will take place. If the systolic BP reaches or exceeds 200 mmHg for more than 5 minutes or the diastolic BP reaches or exceeds 110 mmHg for more than 5 minutes, study physician can administer labetalol 20 mg IV, then 20-80 mg IV every 10 minutes up to dose of 300 mg. A twelve lead EKG, a code cart and defibrillator are available in the room in case of complications. In case of chest pain, chest tightness or other symptoms suggestive of cardiac ischemia, the experiment will be cancelled and a twelve lead EKG will be immediately obtained to rule out angina (ST segment elevation or depression as compared to the baseline EKG). Appropriate treatment will be initiated.

With regards to psychiatric/behavioral side effects, inclusion in this study will be limited to individuals who are between the ages of 18-50. No individuals with psychiatric disorders will be

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recruited into this protocol, on or off medications. Before discharge, study staff will ask specifically about mood changes or suicidal ideation and will refer subjects to the emergency room or to the Clinical Neuroscience Research Unit (CNRU) if warranted as a result of this interview. Upon discharge, subjects will be given the phone numbers of the study physicians. Subjects will be encouraged to call study physicians in case they had any side effects. On the follow up session designed for serum calcium measurements, a study physician will interview subjects in order to make sure that they don't have any side residual effects and/or changes compared to their baseline level before initiating this study.

10.7. Risks associated with questionnaires / interviews: Subjects will have short breaks between questionnaires/interviews. The staff responsible for the data collection has received training in empathetic/non-judgmental techniques for interviewing and will explain to subjects the confidentiality of information. Any identifiable information that is obtained in connection with this study will remain confidential and will be disclosed only with the subject's permission or as required by U.S. or State law. Examples of information that we are legally required to disclose include abuse of a child or elderly person, or certain reportable diseases. All of the information obtained in this study is stored in locked files or password protected computer files. The only ones who have access to this information are the medical personnel and the research staff at the CNRU. When the results of the research are published or discussed in conferences, no information will be included that would reveal subject's identity.

10.8. Risks associated with neurocognitive testing: In order to decrease any frustration with the tests or with subjects' perception of how they are performing on those, the study staff will have clear written instructions. These instructions will clarify how some tests are meant to be challenging. The study staff will also make sure that the cognitive test is not preceded by study procedures, which are cognitively demanding, thus, subjects can focus well.

10.9. Risks associated with privacy/loss of confidentiality (Data Security): Security of user information is of paramount importance to us. Research personnel will not contact any person without appropriate written release of information. All information collected will be kept in password-protected, HIPAA-compliant computers, secured using the best available methods. Information collected during the telephone screening will be discarded in cases when subject is not eligible for a screening in person. Research records will be kept as confidential as possible. Only a code number will identify subject research records. The code number will not be based on any information that could be used to identify subjects (for example, social security number, initials, birth date, etc.). The master list linking names to code numbers will be kept separately from the research data. This master list, as well as all the other research information will be kept in locked files at all times, in Dr. Potenza's /Dr. Angarita's laboratory. Subjects' identity will not be revealed in any reports or publications resulting from this study. Only authorized persons will have access to the information gathered in this study. As part of the study procedures, some

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information about subject's participation will become part of their CMHC medical record. If a subject does not already have a medical record at CMHC, one will be made for their visit. Moreover, if a subject has been a patient at CMHC at any time, his or her previous medical records of other visits or admissions will become available to the researchers and to the staff of the CMHC, as part of the screening, or when the information collected for this research is added into their medical record. Information will be stored as per HIPAA guidelines. All staff has been trained in accordance with HIPAA regulations. Information will not be dispensed to anyone outside of this research project (e.g. , Yale HIC, CMHC, Yale Magnetic Resonance Research Center, and Yale PET Imaging Center) without prior written authorization from the subject. If we see or are told that a child is being abused or neglected or that there is a risk of harm to subject or others, we will disclose this information to the proper authorities. As part of the video (e.g., Skype or Facetime) conference call, subjects will not need to disclose any information about them given that principal investigator will be able to confirm their identity through the camera.

Procedures to minimize risks will be the same for subjects with ADHD. However; there will be no concerns/risks related with Skype or Facetime interview as this will not be required for them.

11. Data and Safety Monitoring Plan: Include an appropriate Data and Safety Monitoring Plan (DSMP) based on the investigator's risk assessment stated below. (Note: the HIC will make the final determination of the risk to subjects.) For more information, see the Instructions, page 24.

a. What is the investigator's assessment of the overall risk level for subjects participating in this study?
Moderate risk

b. If children are involved, what is the investigator's assessment of the overall risk level for the children participating in this study?

c. Include an appropriate Data and Safety Monitoring Plan. Examples of DSMPs are available here http://www.yale.edu/hrpp/forms-templates/biomedical.html for
   i. Minimal risk
   ii. Greater than minimal

d. For multi-site studies for which the Yale PI serves as the lead investigator:
   i. How will adverse events and unanticipated problems involving risks to subjects or others be reported, reviewed and managed?
   ii. What provisions are in place for management of interim results?
   iii. What will the multi-site process be for protocol modifications?

1. Personnel responsible for the safety review and its frequency:

The principal investigator will be responsible for monitoring the data, assuring protocol compliance, and conducting the safety reviews in real time (e.g., on a weekly or bi-weekly basis as part of the Yale Cocaine Clinic rounds). During the review process, the principal investigator

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will evaluate whether the study should continue unchanged, require modification/amendment, or be close to enrollment. Either the principal investigator or the IRB have the authority to stop/ suspend the study or require modifications.

2. The risks associated with the current study are deemed moderate for the following reasons:

We do not view the risks associated with the [^{11}C]-PHNO PET, and/or medication administration component as minimal. Although we have assessed the proposed study as one of moderate risk, the potential exists for anticipated and/or unanticipated adverse events, serious or otherwise, to occur since it is not possible to predict with certainty the absolute risk in any given individual or in advance of first-hand experience with the proposed study methods. Therefore, we provide a plan for monitoring the data and safety of the proposed study as follows:

3. Attribution of Adverse Events:

Adverse events will be monitored for each subject participating in the study and attributed to the study procedures / design by Dr. Marc Potenza, MD, PhD / Dr. Gustavo A. Angarita, MD according to the following categories:

a) Definite: Adverse event is clearly related to investigational procedures(s)/agent(s).
b) Probable: Adverse event is likely related to investigational procedures(s)/agent(s).
c) Possible: Adverse event may be related to investigational procedures(s)/agent(s).
d) Unlikely: Adverse event is likely not to be related to the investigational procedures(s)/agent(s).
e) Unrelated: Adverse event is clearly not related to investigational procedures(s)/agent(s).

4. Plan for Grading Adverse Events:

The following scale will be used in grading the severity of adverse events noted during the study:

A. Mild adverse event
B. Moderate adverse event
C. Severe adverse event

5. Plan for Determining Seriousness of Adverse Events:

Serious Adverse Events: In addition to grading the adverse event, the PI will determine whether the adverse event meets the criteria for a Serious Adverse Event (SAE). An adverse event is considered serious if it:

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1) is life-threatening OR
2) results in in-patient hospitalization or prolongation of existing hospitalization OR
3) results in persistent or significant disability or incapacity OR
4) results in a congenital anomaly or birth defect OR
5) results in death OR
6) based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition, OR
7) adversely affects the risk/benefit ratio of the study

An adverse event may be graded as severe but still not meet the criteria for a Serious Adverse Event. Similarly, an adverse event may be graded as moderate but still meet the criteria for an SAE. It is important for the PI to consider the grade of the event as well as its "seriousness" when determining whether reporting to the HIC is necessary.

6. Plan for reporting Reportable Adverse Events and other unanticipated problems involving risks to subjects or others to the IRB

The principal investigator will report the following types of events to the HIC and the funding agency: a) serious AND unanticipated AND possibly, probably or definitely related events; b) anticipated adverse events occurring with a greater frequency than expected; and c) other unanticipated problems involving risks to subjects or others.

These adverse events or unanticipated problems involving risks to subjects or others will be reported to the HIC within 24-48 hours of it becoming known to the investigator, using the appropriate forms found on the website.

7. Plan for reporting adverse events to co-investigators on the study, as appropriate the protocol's research monitor(s), e.g., industrial sponsor, Yale Cancer Center Data and Safety Monitoring Committee (DSMC), Protocol Review Committee (PRC), DSMBs, study sponsors, funding and regulatory agencies, and regulatory and decision-making bodies.

For the current study, the following individuals, funding, and/or regulatory agencies will be notified (choose those that apply):

☑ All Co-Investigators listed on the protocol.

□ Yale Cancer Center Data and Safety Monitoring Committee (DSMC)

□ National Institutes of Health

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☑ Yale University Radioactive Drug Research Committee (YU RDRC), if applicable

☑ Food and Drug Administration (Yale PET Center Sponsored IND #134,138), if applicable

□ Medical Research Foundation (Grant_____)

☑ The Brain and Behavior Research Foundation

The principal investigator Marc Potenza, MD, PhD will also conduct a review of all adverse events upon completion of every study subject. The principal investigator will evaluate the frequency and severity of the adverse events and determine if modifications to the protocol or consent form are required. DSMP and plan for reporting adverse events will be the same for ADHD subjects.

12. Statistical Considerations: Describe the statistical analyses that support the study design.
Data Analysis: The PET image sets are aligned and re-sliced to yield images in the same planes and spatial system as the MRI images using AAL template. A number of modeling approaches will be taken to quantitate binding potentials (BP_{ND}). Initially, the primary focus will be on reference region approaches, specifically the Simplified Reference Tissue Models (SRTM); i.e., time-activity curves will be fitted with the Simplified reference tissue model to extract binding potentials (BP_{ND}) in various brain regions.

Data Analyses: The primary outcome will be non-displaceable tracer binding potentials ($BP_{ND} = V_T – V_{REF} / V_{REF}$), which are linearly proportional to the density of available $D_{2/3}$ Rs, computed using a simplified reference tissue model (SRTM) utilizing the cerebellum as a reference region. This method has been previously validated. PET data will be reconstructed into images at Yale PET Center with all standard corrections using the appropriate reconstruction protocols and filters. All PET data will be reconstructed with an iterative reconstruction algorithm, MOLAR, designed specifically for the HRRT. MOLAR incorporates attenuation, scatter and deadtime, and subject-motion (detected by the Vicra system), into the forward projection model for the scanner. Emission images are binned into equal frame times (typically 3 minutes). Following image reconstruction, an early summed image (0-12 min) will be registered to the subject's MR image, which will be registered to an MR template so PET can be transformed to a standard (MNI) space. HYPR filtering will be applied to all emission data before analysis. Regions of interest will be the caudate, putamen, ventral striatum (VST), and substantia nigra / ventral tegmental area (SN/VTA). We will perform within-subject comparisons of the relative change in BP_{ND} elicited by calcitriol + Amp vs. placebo + Amp (ΔBP_{ND}), using appropriate parametric (paired t-) or non-parametric (Wilcoxon signed-rank test) tests. Although the proposed sample will enable detection of significant differences of moderate effect sizes (d' = 0.59) with a power of 0.8 and two-tailed alpha of 0.05, the sample will, at a minimum, provide estimates of effect size/power upon which future larger scales studies may be based. This effect size is congruent with preclinical work (Cass, Peters et al. 2012) and with clinical [^{11}C]-(+)-PHNO and [^{11}C]

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Raclopride studies (Shotbolt, Tziortzi et al. 2012). In secondary analyses, we will examine correlations (Pearson's) between cognitive (e.g., CPT-IP and PRLT), psychomotor (e.g., eye blink rate), and subjective (e.g., DEQ and simplified version of the amphetamine interview rating scale) measurements and neuroimaging outcomes/stimulant effects.

Statistical analyses will be the same for subjects with ADHD.

SECTION VI: RESEARCH INVOLVING DRUGS, BIOLOGICS, RADIOTRACERS, PLACEBOS AND DEVICES

If this section (or one of its parts, A or B) is not applicable, state N/A and delete the rest of the section.

A. DRUGS, BIOLOGICS and RADIOTRACERS

1. **Identification of Drug, Biologic or Radiotracer:** What is (are) the **name(s)** of the drug(s) biologic(s) or radiotracer(s) being used? Identify whether FDA approval has been granted and for what indication(s).

Radiotracer ([^{11}C]-PHNO): At the Yale PET center, we have characterized and applied an improved agonist radiotracer for the D2/3 receptor, [^{11}C]-PHNO, demonstrating its excellent test-retest variability in striatum (Gallezot et al., 2014b) and its greater sensitivity to Amp-induced DA release relative to traditional antagonist ligands (i.e., [^{11}C]-raclopride) (Shotbolt et al., 2012). As such, [^{11}C]-PHNO constitutes an optimal tracer for our proposed studies evaluating the effects of calcitriol on stimulant-induced DA release in humans. In addition, our group has considerable experience conducting such experiments with dextro-ampethamine challenges and [^{11}C]-PHNO in humans. [^{11}C]-PHNO has been approved by the RDRC for use in previous human PET protocols at the Yale University PET Center. In addition, an IND was submitted to the FDA for the use of [^{11}C]-PHNO in another protocol at the Yale University PET Center. This IND was approved. We will add current protocol to that IND and we will also submit current protocol for approval by the Yale University RSC.

Calcitriol: The active form of vitamin D_3, is approved for the treatment of hypocalcemia in patients on chronic renal dialysis, hypocalcemia in hypoparathyroidism/pseudohypoparathyroidism, vitamin D resistant rickets, and secondary hyperparathyroidism in patients with moderate to severe chronic kidney disease not yet on dialysis. Another approved indication is psoriasis (e.g., topically). An off-label use is Vitamin D-dependent rickets type 1/pseudovitamin D deficiency rickets (PDDR). There have been phase I and phase II clinical studies on calcitriol for cancer (Beer, Munar et al. 2001, Beer, Javle et al. 2007, Beer, Ryan et al. 2007, Ramnath, Daignault-Newton et al. 2013). In terms of the oral administration, calcitriol comes in capsules of 0.5 mcg. The US labeling recommends doses up to 1 mg per day (e.g., for hypocalcemia in patients on chronic renal dialysis) or up to 2 mcg per day (e.g.,

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for hypocalcemia in hypoparathyroidism/pseudohypoparathyroidism). To the best of our knowledge, calcitriol has never been tested/used in human subjects as an adjunct to stimulant medications.

Dextro-amphetamine (Dexedrine): Is a centrally acting sympathomimetic amine that promote release of catecholamines (primarily dopamine and norepinephrine) from their storage sites in the presynaptic nerve terminals. Another mechanism includes its ability to block the reuptake of catecholamines. It has ben approved for treatment of Narcolepsy (e.g., doses up to 60 mg per day) and Attention Deficit Hyperactivity Disorder (ADHD) (e.g., doses up to 40 mg per day). It comes in tablets of 5, 7.5, 10, 15, 20, and 30 mg.

Radiotracer, calcitriol, and Dexedrine will be the same for ADHD subjects.

All protocols which utilize a drug, biologic or radiotracer **not** approved by, but regulated by, the FDA, or a radiotracer regulated by the RDRC, must provide the following information:

a. What is the Investigational New Drug (IND) **number** assigned by the FDA?
b. IND # for ^{11}C-PHNO is 134,138
c. Who holds the IND?
   Yale University PET Imaging Center

c. All protocols which utilize a radiotracer not approved by, but regulated by the FDA must provide the IND number: ____134,138_____
Alternatively, use of the investigational radiotracer may be under RDRC/RSC oversight: (check if appropriate)_____

For all investigational radiotracers, attach a copy of the RDRC/RSC application (for radioisotopes used in the PET Center, PET Center personnel may complete this step)
Go to http://rsc.med.yale.edu/login.asp?url=myApps.asp. When you have logged in, complete the application and attach a copy to this submission.

Alternatively, an **exemption from IND filing requirements** may be sought for a clinical investigation of a drug product that is lawfully marketed in the United States. If there is no IND and an exemption is being sought, review the following categories and complete the category that applies (*and delete the inapplicable categories)*:

Exempt Category 1 for the calcitriol and dextro-amphetamines
The clinical investigation of a drug product that is lawfully marketed in the United States can be exempt from IND regulations if all of the following are yes:

i. The intention of the investigation is NOT to report to the FDA as a well-controlled study in support of a new indication for use or to be used to support any other significant change in the labeling for the drug. ☒ Yes ☐ No
ii. The drug that is undergoing investigation is lawfully marketed as a prescription drug product, and the intention of the investigation is NOT to support a significant change in the advertising for the product. ☒ Yes ☐ No
iii. The investigation does NOT involve a route of administration or dosage level or use in populations or other factor that significantly increases the risks (or decreases the acceptability of the risks)

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associated with the use of the drug product. ☒ Yes ☐ No

iv. The investigation will be conducted in compliance with the requirements for institutional (HIC) review and with the requirements for informed consent of the FDA regulations (21 CFR Part 50 and 21 CFR Part 56). ☒ Yes ☐ No

v. The investigation will be conducted in compliance with the requirements regarding promotion and charging for investigational drugs. ☒ Yes ☐ No

Exempt Category 2 (all items i, ii, and iii must be checked to grant a category 2 exemption)

☐ i. The clinical investigation is for an *in vitro* diagnostic biological product that involves one or more of the following (check all that apply):

- ☐ Blood grouping serum
- ☐ Reagent red blood cells
- ☐ Anti-human globulin

☐ ii. The diagnostic test is intended to be used in a diagnostic procedure that confirms the diagnosis made by another, medically established, diagnostic product or procedure; and

☐ iii. The diagnostic test is shipped in compliance with 21 CFR §312.160.

Exempt Category 3

☐ The drug is intended solely for tests in vitro or in laboratory research animals if shipped in accordance with 21 CFR 312.60

Exempt Category 4

☐ A clinical investigation involving use of a placebo if the investigation does not otherwise require submission of an IND.

Background Information: Provide a description of previous human use, known risks, and data addressing dosage(s), interval(s), route(s) of administration, and any other factors that might influence risks. If this is the first time this drug is being administered to humans, include relevant data on animal models.

[^{11}C]-PHNO has been used in several PET centers (Ginovart, Willeit et al. 2007). It was initially developed at the PET Center, University of Toronto, and the safety of its use in humans has been demonstrated.

[^{11}C]-PHNO has been used extensively used in many clinical trials. A survey of the *ClinicalTrials.gov* website shows 12 completed trials, 5 recruiting trials, and 1 active clinical trial with [^{11}C]-PHNO investigating a variety of behaviors and disorders: alcohol drinking, addictive behavior, behavioral symptoms, cocaine-related disorders, compulsive behavior, depression, depressive disorder, drinking behavior, impulsive behavior, mental disorders, mood disorders, obsessive-compulsive disorder, psychotic disorders, schizophrenia studies, schizophrenia spectrum

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and other psychotic disorders, psychological stress, substance-related disorders, and tobacco use disorder.

The use of [^{11}C]-PHNO in PET imaging in human was first reported in 2006 (Willeit, Ginovart et al. 2006). No relevant abnormalities in blood pressure, heart rate, or ECG were reported at any time in the study. There also were no relevant findings in physical or neurological exams or in routine blood and urine analyses during the study. However nausea was reported approximately 2–3 min after tracer injection, which subsided rapidly 2–3 min later. This effect was attributed to the transient high [^{11}C]-PHNO concentration resulting from the bolus injection (Willeit, Ginovart et al. 2006).

Four hundred and eighty six [^{11}C]-PHNO human PET scans have been reviewed to assess the side effect profile and their recurrence (Mizrahi, Houle et al. 2010). The injected mass ranged from 0.85 to 5.56 μg, with a mean of 2.30 μg. Injected doses ranged from 0.01 to 0.08 μg/kg, with a mean of 0.03 μg/kg (SE, 0.0004 μg/kg). Medical attention was not required for any of the reviewed cases, and no side effects were reported in 84.6% of the scans reviewed. Nausea was reported in 14.3%, while vomiting was reported in 1.1%. These symptoms seem to have arose 3–5 min after the injection and subsided within 7–12 min in all cases. The review concluded that [^{11}C]-PHNO dose of ≤ 0.029 μg/kg is highly unlikely to produce any side effects. Furthermore, there was no relationship between dose and nausea for [^{11}C]-PHNO doses of ≤ 0.029 μg/kg.

We have now administered [^{11}C]-PHNO to human subjects over 250 times at the Yale PET Center. All injected doses were limited to ≤ 0.03 μg/kg. These studies included smokers, cocaine users, pathological gamblers, schizophrenia subjects, and healthy controls. One unexpected adverse effect (unresponsiveness for approximately one minute) 12 minutes post-injection of [^{11}C]-PHNO was reported and was likely due to underlying cardiac history that was possibly exacerbated by study procedures. A comprehensive review of vital signs data was conducted on all subjects injected with [^{11}C]-PHNO at the Yale University PET Center and showed no correlation or relationship of any kind between injection of [^{11}C]-PHNO and systolic blood pressure, diastolic blood pressure, or heart rate measures.

Adverse events were observed in studies which involved administration of amphetamine in addition to [^{11}C]-PHNO. The typical protocol involved a morning [^{11}C]-PHNO administration, followed by oral amphetamine, followed by a second PHNO injection, starting 3 hours post-amphetamine. Because nausea is attributed to the transient high [^{11}C]-PHNO concentration 2–3 min after the bolus injection of the tracer (Willeit, Ginovart et al. 2006), the Yale PET Center administers [^{11}C]-PHNO as a bolus over a period of 5 minutes, *i.e.*, at a PHNO dose rate of 7 ng/min for the average 70 kg subject. Administration of [^{11}C]-PHNO dose at this rate is about 5 times slower than lowest rate reported in the clinical study involving i.v. administration of PHNO (Coleman, Quinn et al. 1990).

For the studies involving amphetamine, 30 research subjects participated, of whom 21 received two [^{11}C]-PHNO injections each, and 9 subjects received a single [^{11}C]-PHNO injection, for a total of 51 injections. Five subjects received a single injection of PHNO and did not receive amphetamine, and none experienced an adverse event. Twenty five subjects received both PHNO and amphetamine. No adverse events were experienced from the time immediately following the first PHNO injection (baseline) until amphetamine administration. Adverse events occurred in 6 of 25 subjects (24%), after amphetamine dosing. In 4 of the 6 subjects, the adverse event began 30-90 minutes after dosing with

amphetamine (4 of 25; 16%), but before the second PHNO administration. In 2 of the 6 subjects, the adverse event began approximately 3 hours after dosing with amphetamine (2 of 25, 8%), i.e., after the second [^{11}C]-PHNO. Adverse events reported on these 6 subjects were nausea/vomiting, dizziness, headache, epigastric pain, insomnia, psychosis, cardiac arrhythmia, hypertention, and psychosis.

Calcitriol: The frequency for potential side effects (e.g., please, see detailed description for these on section 9.5) of calcitriol is not well defined. There are certain circumstances that can increase the probabilities of adverse events, such as impaired kidney function, impaired liver function, high phosphorus, interaction with drugs/supplements that can increase calcium levels or that can cause complications in the setting of elevated calcium levels (e.g., cardiac glycosides), and vulnerability/recent history of kidney stones. There are studies using weekly doses of calcitriol among patients with malignancies. Each cycle consisted of 4 weekly administrations followed by 4 weeks of observation. Patients who tolerated each cycle proceeded to the next cycle up to weekly doses as high as 2 mcg/kg. This study revealed 8 instances of self-limited hypercalcemia and did not reveal toxicities requiring any medical intervention. As part of this study, authors proposed doses of 0.5 mcg/kg for phase II studies (Beer, Munar et al. 2001). There are also pharmacokinetic studies among healthy controls without any malignancies using intravenous or oral doses of 4 mcg and which did not show any significant side effects (Brandi, Egfjord et al. 2002). There are also studies among healthy controls using it at doses of 1.5 mcg/meter^2 of body surface area (N = 10) (Bianchi, Ardissino et al. 1999) or at doses of 3 mcg per day (N = 11) (Broadus, Erickson et al. 1984, Erickson, Cooper et al. 1984), which did not show significant side effects. The latter studies administered it for 10 consecutive days.

Subjects who have circumstances increasing risks of potential side effects will be excluded. In addition, we will monitor calcium levels as part of the study and we will have in place preventive actions that could decrease risks of potential side effects (e.g., modification of diet, higher intake of water, avoiding medications that could interact with calcium or calcitriol). We also preferred to split the total dose of 3 mcg in two different administrations of 1.5 mcg for ease of administration (e.g., three capsules will be easier than six), to maximize chances that [^{11}C]PHNO scans are conducted after calcitriol has worked (e.g., Onset of action after 2 hours; maximum effect after 10 hours; duration of effect is 3 to 5 days), and to maximize chances of subjects tolerating it.

Dexedrine: Most a complete list of potential side effects, please see section 9.6. Dexedrine is Clinically used in humans for treatment of ADHD and Narcolepsy at daily doses ranging from 5 to 60 mg. We have also experienced using it for our PET imaging studies with doses of 0.4 and 0.5 mg/kg. Proposed dose for this study of 0.3 mg/kg will be equivalent to 30 mg in a subject whose weight is 100 kg, thus within the doses FDA approved for therapeutic purposes. In terms of conditions increasing risks of side effects, subjects who have those will be excluded from the study. We will also monitor subjects throughout the study in order to early capture presence of any significant side effects (e.g., cardiovascular, psychiatric, or of any other nature). We will use the oral route due to the greater safety and ease of administration.

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Taking into account that there are no studies administering calcitriol the same day as dextro-amphetamines, we will use doses of each drug lower than the doses we have used before.

3. **Source:** a) Identify the source of the drug or biologic to be used.

[^{11}C]-PHNO prepared at the Yale PET Center.
Calcitriol capsules and Dexedrine tablets will be provided by the CMHC pharmacy.

b) Is the drug provided free of charge to subjects? ☒ Yes ☐ No
If yes, by whom?

4. **Storage, Preparation and Use:** Describe the method of storage, preparation, stability information, and for parenteral products, method of sterilization and method of testing sterility and pyrogenicity.

Check applicable Investigational Drug Service utilized:

☐ **YNHH IDS**	☐ **Yale Cancer Center**
☒ **CMHC Pharmacy**	☐ **West Haven VA**
☒ **PET Center**	☐ **None**
☐ **Other:**	

Note: If the YNHH IDS (or comparable service at CMHC or WHVA) will not be utilized, explain in detail how the PI will oversee these aspects of drug accountability, storage, and preparation.

Due to the short half-life, PET drugs are prepared and formulated immediately before administration, and therefore there are no issues with storage or stability. PET drug products are stored at room temperature and are stable for at least 60 min after preparation.

The preparation of sterile PET drug products is validated prior to human use. Sterility is achieved by passing the PET drug product through a 0.22 micron membrane filter during the last step in the formulation process. Prior to release for administration, a bubble point test is performed on the membrane filter used for terminal sterilization in order to validate and verify its integrity during the filtration process. Due to the short half-life, a sample of the PET drug product is tested for sterility after administration for further confirmation. The level of endotoxin in each batch of the final PET drug product is determined quantitatively prior to release for administration using the FDA approved Charles River Laboratory's Portable Testing System (Endosafe®-PTS).

[^{11}C]-PHNO will be prepared at the Yale PET Center in high radiochemical purity and high specific activity in accordance with our local Chemistry Manufacturing & Control (CMC) procedures and quality specifications described in local Drug Master File (DMF).

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procedures and quality specifications described in local Drug Master File (DMF). Briefly, [^{11}C]propionyl chloride is prepared by reaction of [^{11}C]CO2 with ethylmagnesium bromide, followed by treatment with phthaloyl dichloride. Then, *N*-acylation of the naphthoxazine precursor with [^{11}C]propionyl chloride, followed by reduction of the resulting [^{11}C]amide intermediate, yields [^{11}C]-PHNO. The resulting PET drug product is purified first by semi-preparative HPLC, followed by solid-phase extraction to remove the HPLC buffer mixture. Finally [^{11}C]-PHNO is formulated in <10% ethanolic saline solution (USP), and the resulting PET drug product is then passed through a 0.22 micron sterile membrane filter for terminal sterilization and collected in a sterile pyrogen free collection vial to afford a formulated I.V. solution ready for dispensing and administration.

5. **Use of Placebo: X Not applicable to this research project**
   If use of a placebo is planned, provide a justification which addresses the following:
   1. Describe the safety and efficacy of other available therapies. If there are no other available therapies, state this.
   b. State the maximum total length of time a participant may receive placebo while on the study.
   c. Address the greatest potential harm that may come to a participant as a result of receiving placebo.
   d. Describe the procedures that are in place to safeguard participants receiving placebo.

6. **Use of Controlled Substances:**
   Will this research project involve the use of controlled substances in human subjects?
   ☒ Yes ☐ No *See HIC Application Instructions to view controlled substance listings*.

   If yes, is the use of the controlled substance considered:
   ☐ Therapeutic: The use of the controlled substance, within the context of the research, has the potential to benefit the research participant.
   ☒ Non-Therapeutic: *Note, the use of a controlled substance in a non-therapeutic research study involving human subjects may require that the investigator obtain a Laboratory Research License. Examples include controlled substances used for basic imaging, observation or biochemical studies or other non-therapeutic purposes. See Instructions for further information.*

   Dextro-amphetamine (0.3 mg/kg) will be given to each subject, by mouth, each experiment day, 2.5 hours after administration of calcitriol or placebo.

7. **Continuation of Drug Therapy After Study Closure X Not applicable to this project**
   Are subjects provided the opportunity to continue to receive the study drug(s) after the study has ended?
   ☐ Yes If yes, describe the conditions under which continued access to study drug(s) may apply as well as conditions for termination of such access.

   ☒ No If no, explain why this is acceptable.

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SECTION VII: RECRUITMENT/CONSENT AND ASSENT PROCEDURES

1. **Targeted Enrollment: Give the number of subjects:**
   a. targeted for enrollment at Yale for this protocol__60 (30 per cohort)_
   b. If this is a multi-site study, give the total number of subjects targeted across all sites__Single-site_

2. **Indicate recruitment methods below.** Attach copies of any recruitment materials that will be used.

☒ Flyers	☒ Internet/Web Postings	☒ Radio
☒ Posters	☐ Mass E-mail Solicitation	☐ Telephone
☐ Letter	☐ Departmental/Center Website	☐ Television
☐ Medical Record Review*	☐ Departmental/Center Research Boards	☒ Newspaper
☐ Departmental/Center Newsletters	☐ Web-Based Clinical Trial Registries	
☒ YCCI Recruitment database	☒ Clinicaltrials.gov Registry (do not send materials to HIC)	

☒ Other (describe): Word of mouth referrals; YCCI Facebook/Twitter

***Requests for medical records should be made through JDAT as described at** http://medicine.yale.edu/ycci/oncore/availableservices/datarequests/datarequests.aspx

3. **Recruitment Procedures:**
   a. Describe how potential subjects will be identified.
   Subject will be recruited through the methods listed above such as flyers, radio adds, word of mouth referrals (see written material attached to protocol).

   b. Describe how potential subjects are contacted.
   Potential subjects will be encouraged to contact our study recruitment line for inclusion in our study.

   c. Who is recruiting potential subjects?

Subjects are recruited through our recruitment phone line. Subjects are asked to call in on our dedicated recruitment line. A member of our research staff will describe the study to participants who call, answer any questions the potential subjects has, and then complete a phone screening questionnaire to determine the subjects eligibility for an in-person screening visit. Subjects who are considered eligible based on this phone screening will be identified as potential participants therefore invited for the screening in person.

If an individual appears to meet enrollment criteria and is interested in participating, a face-to-face interview is conducted by the study staff and study physician. A release of information is obtained for review of any available historical and clinical data. A written authorization form is also obtained from each subject, permitting the research team to use, create, or disclose the subject's PHI for research purposes. The nature of the project, procedures, relative risks and benefits, and alternatives to participation in the project are discussed with the individual. Following this discussion, the individual is given a copy of the consent form to review, and any questions are answered. The process of informed consent will be obtained in accordance with

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local IRB standards by study personnel who have participated in institutionally approved training in human subject protection. Upon obtaining voluntary, written, informed consent, medical and psychiatric screening procedures will be used to confirm study eligibility. Subjects are free to discontinue their participation in the research at any time.

4. Screening Procedures

a. Will email or telephone correspondence be used to screen potential subjects for eligibility prior to the potential subject coming to the research office? ☒ Yes ☐ No

b. If yes, identify below all health information to be collected as part of screening and check off any of the following HIPAA identifiers to be collected and retained by the research team during this screening process.

HEALTH INFORMATION TO BE COLLECTED:

HIPAA identifiers:

☒Names

☒ All geographic subdivisions smaller than a State, including: street address, city, county, precinct, zip codes and their equivalent geocodes, except for the initial three digits of a zip code if, according to the current publicly-available data from the Bureau of the Census: (1) the geographic unit formed by combining all zip codes with the same three initial digits contains more than 20,000 people, and (2) the initial three digits of a zip code for all such geographic units containing 20,000 or fewer people is changed to 000.

☒ Telephone numbers

☐ Fax numbers

☒ E-mail addresses

☐ Social Security numbers

☐ Medical record numbers

☐ Health plan beneficiary numbers

☐ Account numbers

☒ All elements of dates (except year) for dates related to an individual, including: birth date, admission date, discharge date, date of death, all ages over 89 and all elements of dates (including year) indicative of such age, except that such ages and elements may be aggregated into a single category of age 90 or older

☐ Certificate/license numbers

☐ Vehicle identifiers and serial numbers, including license plate numbers

☐ Device identifiers and serial numbers

☐ Web Universal Resource Locators (URLs)

☐ Internet Protocol (IP) address numbers

☐ Biometric identifiers, including finger and voice prints

☐ Full face photographic images and any comparable images

☐ Any other unique identifying numbers, characteristics, or codes

Recruitment and screening procedures are the same for ADHD subjects.

5. Assessment of Current Health Provider Relationship for HIPAA Consideration:

Does the Investigator or any member of the research team have a direct existing clinical relationship with any potential subject?

☐ Yes, all subjects

☐ Yes, some of the subjects

☒ No

If yes, describe the nature of this relationship.

6. **Request for waiver of HIPAA authorization:** (When requesting a waiver of HIPAA Authorization for either the entire study, or for recruitment purposes only. Note: if you are collecting PHI as part of a phone or email screen, you must request a HIPAA waiver for recruitment purposes.)

Choose one:

☐ For entire study

☒ For recruitment purposes only

☐ For inclusion of non-English speaking subject if short form is being used **and a translated HIPAA research authorization form is not available on the University's HIPAA website**

i. Describe why it would be impracticable to obtain the subject's authorization for use/disclosure of this data;

The information we are collecting over the phone is required prior to the subject being considered for an in-person screening appointment.

ii. If requesting a waiver of **signed** authorization, describe why it would be impracticable to obtain the subject's signed authorization for use/disclosure of this data;

By signing this protocol application, the investigator assures that the protected health information for which a Waiver of Authorization has been requested will not be reused or disclosed to any person or entity other than those listed in this application, except as required by law, for authorized oversight of this research study, or as specifically approved for use in another study by an IRB.

Researchers are reminded that unauthorized disclosures of PHI to individuals outside of the Yale HIPAA-Covered entity must be accounted for in the "accounting for disclosures log", by subject name, purpose, date, recipients, and a description of information provided. Logs are to be forwarded to the Deputy HIPAA Privacy Officer.

7. **Required HIPAA Authorization:** If the research involves the creation, use or disclosure of protected health information (PHI), separate subject authorization is required under the HIPAA Privacy Rule. Indicate which of the following forms are being provided:
   ☒ Compound Consent and Authorization form
   ☐ HIPAA Research Authorization Form

8. **Consent Personnel:** List the names of all members of the research team who will be obtaining consent/assent.

Consenting personnel is listed in IRES IRB.

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9. Process of Consent/Assent: Describe the setting and conditions under which consent/assent will be obtained, including parental permission or surrogate permission and the steps taken to ensure subjects' independent decision-making.

All subjects will be consented through a multi-step consent process. Subjects are first described the study in detail over the phone during the phone screen. Health information such as medical, psychiatric, and substance use history, questions about presence of metal in their bodies, and information about current medications. If subjects are deemed appropriate from the phone screen, they are then invited to come the in-person screening appointment on the Clinical Neuroscience Research Unit. Consenting personnel will meet with the subject in person, explain both the consent form and the protocol and answer any questions the subject might have. The subject will be given a copy of the consent to read, at their leisure, prior to signing. Once the subject has signed the consent form the screening visit procedures will begin. This will take place at the Clinical Neuroscience Research Unit of CMHC.

Remote screening option: Subjects who pass telephone screening will be invited to a more in-depth screening to determine study eligibility (as listed in section on inclusion/exclusion criteria). Potential subjects who pass the phone screen portion will have the option to do the consenting screening portion remotely. They will be sent two copies of the consent form and a release of information to review at their leisure through a method that is most convenient for them (i.e., email, fax, or mail). This way subjects have time to review the consent prior to signing and they have their own copy to follow along and ask questions during the consenting phone call. If they agree to participate, subjects can keep one copy of the consent and hand-sign, date, and return the other copy along with a signed released of information back to our research office either by fax, email, or mail in a prepaid envelope. When the consent is also signed by a member of the research team, it will be filed in the subject's research record. Using tele-health platforms (i.e., Zoom) the researcher and subject will thoroughly review and sign the consent form, complete a structured clinical interview (SCID) by a member of the research team, and a medical history and psychiatric interview by a study physician. This will take up to two hours to complete in total. If deemed eligible by initial Zoom call and interviews, potential subjects will be asked to complete the rest of the study procedures as detailed in the protocol.

10. Ev**aluation of Subject(s) Capacity to Provide Informed Consent/Assent:** Indicate how the personnel obtaining consent will assess the potential subject's ability and capacity to consent to the research being proposed.

We do not plan to recruit subjects with limited decision-making capacity. Potential subjects will undergo a phone interview and a face-to-face interview. At that time, the person obtaining consent will meet the study subject, review the informed consent form, explain the purpose of the study and risks associated with participation, and will be available for questions. Study staff is trained to assess the subject's understanding of pertinent information given to them and whether or not subjects can appreciate the implications of their decision. To ensure that the study subject understands the study, the subject will be asked questions about the study

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procedures and the risks associated with participation. If any concern arises that the study subject did not fully understand the study, the person obtaining consent may decide that the subject is not suitable for participation. This process generally takes about 1 hour. If the subject is still interested, after all questions have been answered, the person obtaining consent will ask the subject to sign the informed consent form.

11. Documentation of Consent/Assent: Specify the documents that will be used during the consent/assent process. Copies of all documents should be appended to the protocol, in the same format that they will be given to subjects.

Compound consent and authorization form.

Process of consent form, evaluation of subject's capacity to provide informed consent, and documentation of consent will be the same for ADHD subjects.

12. Non-English Speaking Subjects: Explain provisions in place to ensure comprehension for research involving non-English speaking subjects. If enrollment of these subjects is anticipated, translated copies of all consent materials must be submitted for approval prior to use.

Non-English speaking healthy subjects nor ADHD subjects will not be enrolled in this study.

12(a) As a limited alternative to the above requirement, will you use the short form* for consenting process if you unexpectedly encounter a non-English speaking individual interested in study participation and the translation of the long form is not possible prior to intended enrollment?
YES ☐ NO ☐

Note* If more than 2 study participants are enrolled using a short form translated into the same language, then the full consent form should be translated into that language for use the next time a subject speaking that language is to be enrolled.

Several translated short form templates are found on our website at: http://www.yale.edu/hrpp/forms-templates/biomedical.html. If the translation of the short form is not available on our website, then the translated short form needs to be submitted to the IRB office for approval via amendment prior to enrolling the subject. ***Please review the guidance and presentation on use of the short form available on the HRPP website.***

If using a short form without a translated HIPAA Research Authorization Form, please request a HIPAA waiver in the section above.

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13. Consent Waiver: In certain circumstances, the HIC may grant a waiver of signed consent, or a full waiver of consent, depending on the study. If you will request either a waiver of consent, or a waiver of signed consent for this study, complete the appropriate section below.

☐ **Not Requesting a consent waiver**
☒ **Requesting a waiver of signed consent**
☐ **Requesting a full waiver of consent**

A. Waiver of **signed** consent: (Verbal consent from subjects will be obtained. **If PHI is collected, information in this section must match Section VII, Question 6**)
X Requesting a waiver of signed consent for Recruitment/Screening only

If requesting a waiver of signed consent, please address the following:
a. Would the signed consent form be the only record linking the subject and the research?
☒ Yes ☐ No
b. Does a breach of confidentiality constitute the principal risk to subjects?
☐ Yes ☒ No

OR

c. Does the research activity pose greater than minimal risk?
☐ Yes ***If you answered yes, stop. A waiver cannot be granted.*** Please note: Recruitment/screening is generally a minimal risk research activity
☐ No

AND

d. Does the research include any activities that would require signed consent in a non-research context? ☐ Yes ☐ No

☐ **Requesting a waiver of signed consent for the Entire Study (**Note that an information sheet may be required.)

If requesting a waiver of signed consent, please address the following:
a. Would the signed consent form be the only record linking the subject and the research?
☐ Yes ☐ No
b. Does a breach of confidentiality constitute the principal risk to subjects?
☐ Yes ☐ No

OR

c. Does the research pose greater than minimal risk? ☐ Yes ***If you answered yes, stop. A waiver cannot be granted.*** ☐ No

AND

d. Does the research include any activities that would require signed consent in a non-research context? ☐ Yes ☐ No

B. **Full waiver** of consent: (No consent from subjects will be obtained for the activity.)
☐ **Requesting a waiver of consent for Recruitment/Screening only**
a. Does the research activity pose greater than minimal risk to subjects?

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☐ Yes ***If you answered yes, stop. A waiver cannot be granted.*** Please note: Recruitment/screening is generally a minimal risk research activity
☐ No
b. Will the waiver adversely affect subjects' rights and welfare? ☐ Yes ☐ No
c. Why would the research be impracticable to conduct without the waiver?
d. Where appropriate, how will pertinent information be returned to, or shared with subjects at a later date?

☐ **Requesting a full waiver of consent for the <u>Entire Study</u> (Note: If PHI is collected, information here must match Section VII, question 6.)**

If requesting a full waiver of consent, please address the following:

a. Does the research pose greater than minimal risk to subjects?
☐ Yes ***If you answered yes, stop. A waiver cannot be granted.***
☐ No
b. Will the waiver adversely affect subjects' rights and welfare? ☐ Yes ☐ No
c. Why would the research be impracticable to conduct without the waiver?
d. Where appropriate, how will pertinent information be returned to, or shared with subjects at a later date?

SECTION VIII: PROTECTION OF RESEARCH SUBJECTS

Confidentiality & Security of Data:

a. What protected health information (medical information along with the HIPAA identifiers) about subjects will be collected and used for the research?

Subjects who decide to participate in the study will have a medical record at the Connecticut Mental Health Center (CMHC). If subjects already have a medical record at CMHC, some information about their participation in the study will be included here. If they do not have a medical record at CMHC, one will be made for their visit. The information that will be entered into this medical record will include: name, date of birth, dates of workup in CMHC/scan at the PET center, phone number, address, medical history, physical exams, electrocardiograms, and blood work. Subject's name, date of birth, date of scans, and phone number will not be used in the analysis neither in the presentation of the results. Results of the MRI/PET scans, results from computerized assessments, and results from paper and pencil questionnaires will be stored on secure servers for purposes of data analysis.

b. How will the research data be collected, recorded and stored?

Research data will be collected over the phone, by paper and pencil questionnaires, by interviews, by neurocognitive assessments, by PET scanning, MR scanning, computerized assessments, and

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laboratory work. This information will be recorded/transferred in each subject's Case Report Form and on a password protected University computer servers that are kept in locked offices.

c. How will the digital data be stored? ☐ CD ☐ DVD ☐ Flash Drive ☐ Portable Hard Drive ☒ Secured Server ☒ Laptop Computer ☒ Desktop Computer ☐ Other

d. What methods and procedures will be used to safeguard the confidentiality and security of the identifiable study data and the storage media indicated above during and after the subject's participation in the study?

All portable devices must contain encryption software, per University Policy 5100. *If there is a technical reason a device cannot be encrypted please submit an exception request to the Information Security, Policy and Compliance Office by clicking on url* http://its.yale.edu/egrc or email it.compliance@yale.edu

All of the information obtained in this study is stored in locked files or password protected and encrypted computer files. The only ones who have access to this information are the medical personnel and the research staff at the CNRU or the Yale PET Center.

Identifiable research data are stored on a secure database located on the internal PET Center Network. The PET network is protected by a Cisco PIX firewall operated by ITS. All research data are backed up nightly to a Dell PV-136T library wit 4 IBM Ultrium-TD2 tape drives using the backup software Legato Networker 7.3 from EMC. Human subjects enrolled in the study are assigned a subject-specific random identifier. Subject identifiers and the means to link the subject names and codes with the research data are stored in separate locations within the database. The software of the database limits the ability to connect the random identifier to the actual subject identification information to research team members only. Access to the database is password protected and each research team member is required to have a unique ID and password to gain access to the database. Authorized users employ their netid and authentication is performed using Yale's central authentication server. Users always access research data through the random identifier only. Direct identifiers belonging to subjects who withdraw from the study, will be stripped from the key.

Results are published as group data without the use of characteristics that would identify individual subjects. We quote information only by number in conference discussions, scientific reports, or publications, in order to maintain anonymity.

Procedures to ensure confidentiality follow the policies of Yale School of Medicine.

Any identifiable information obtained in connection with this protocol remains confidential. It is disclosed only with the subject's prior authorization or as required by U.S. and State law. Information that we are legally required to disclose includes abuse of a child or elderly person, or certain reportable infectious disease.

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Information is discussed among research personnel within a clinical setting. Authorized representatives of the Yale Human Investigation Committee or the FDA may review records for auditing purpose.

If the subject provides written consent to release research information to an insurer, employer, or other person, then we may not withhold that information.

e. What will be done with the data when the research is completed? Are there plans to destroy the identifiable data? If yes, describe how, by whom and when identifiers will be destroyed. If no, describe how the data and/or identifiers will be secured.

This information will be kept in a locked office during five years. Afterwards, identifying information will be discarded, but the other research data is kept indefinitely. Paper and electronic data will be destroyed by research assistants and study personnel at Yale University. Paper media will be destroyed by shredding and electronic media will be destroyed by zeroing, degaussing, or physical destroying as applied to the medium. De-identified MRI/PET imaging data will be kept for a minimum of 7 years.

f. Who will have access to the protected health information (such as the research sponsor, the investigator, the research staff, all research monitors, FDA, Yale Cancer Center Data and Safety Monitoring Committee (DSMC), SSC, etc.)? (please distinguish between PHI and de-identified data)

The research staff at Yale University will have access to PHI. The research Sponsor and the HIC could have access to de-identified data. The HIC can have access to identified data for auditing purposes.

g. If appropriate, has a Certificate of Confidentiality been obtained? A Certificate of Confidentiality for this study has been provided through the NIH.

h. Are any of the study procedures likely to yield information subject to mandatory reporting requirements? (e.g., HIV testing – reporting of communicable diseases; parent interview -incidents of child abuse, elderly abuse, etc.). Please verify to whom such instances will need to be reported.

In the case that elder or child abuse is discovered this will be reported to Connecticut Department of Social Services; Protective Services for the Elderly and/or The Child Abuse and Neglect Care line.

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SECTION IX: POTENTIAL BENEFITS

Potential Benefits: Identify any benefits that may be reasonably expected to result from the research, either to the subject(s) or to society at large. (Payment of subjects is not considered a benefit in this context of the risk benefit assessment.)

Subjects will receive a thorough medical / psychiatric evaluation at no cost to themselves, as well as benefit from the knowledge that their participation in such studies may help others in the future by identifying the neurochemical basis of calcitriol function in brain, and pointing to potential augmentation strategies for the treatment of ADHD. Otherwise, there are no direct benefits to individuals participating in this research. Expanding the current understanding on neurocognitive effects of vitamin D use could benefit society, and consequently better methods of treatment could be designed.

SECTION X: RESEARCH ALTERNATIVES AND ECONOMIC CONSIDERATIONS

1. **Alternatives:** What other alternatives are available to the study subjects outside of the research?

Participants can choose not to participate in this research study.

2. **Payments for Participation (Economic Considerations):** Describe any payments that will be made to subjects, the amount and schedule of payments, and the conditions for receiving this compensation.

Subjects will be paid $25 for completion of the screening visit, $200 for completion of each [^{11}C]-PHNO scan ($800 total), $25 for the completion of the structural MRI, $25 for completion of the first follow up visit, and $25 upon completion of the second follow up visit for a total compensation of up to $900. In the event that a PET scan day is cancelled for reasons outside of your control, you will receive a minimum payment of $50 per cancelled scan.

ADHD inpatient subjects will have a $500 bonus for completion of inpatient study procedures thus their total compensation could be up to $1,400.

Participants will be paid $20 by referring a potential participant who enrolls in the study (i.e., passes telephone screening and signs consent forms). The referral fee will be provided once the referred participant enrolls in the study. In order to enroll, the referred participant will call the study team instead of study team taking this initiative.

Payments will be made in loadable increments after completing individual study procedures via a Bank of America debit card that will be mailed to subjects after completing the screening process.

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3. **Costs for Participation (Economic Considerations):** Clearly describe the subject's costs associated with participation in the research, and the interventions or procedures of the study that will be provided at no cost to subjects.

Subjects will incur no costs for participation in this research outside of potential costs for parking or transportation that may be reimbursed.

In addition, on full-day study sessions for PET scanning, the participant will either be provided or reimbursed for a meal or snacks within twenty dollars and with a receipt.

4. **In Case of Injury:** This section is required for any research involving more than minimal risk, and for minimal risk research that presents the potential for physical harm (e.g., research involving blood draws).

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a. Will medical treatment be available if research-related injury occurs?
b. Where and from whom may treatment be obtained?
c. Are there any limits to the treatment being provided?
d. Who will pay for this treatment?
e. How will the medical treatment be accessed by subjects?

Subjects will be assisted by research staff in obtaining medical care through information and/or referral if injury occurs during their participation in this study. Subjects will be responsible for the cost of any medical care required.

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