Abbreviated Title: cAMP Signaling Affected by Ketamine

NIH IRB #: 000824

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Title: [^{11}C]-(*R*)-Rolipram to Measure cAMP Signaling Before and After Ketamine

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Investigational Agents

Drug Name:	[^{11}C](*R*)-rolipram
IND Number:	73149
Sponsor:	NIMH IRP
Manufacturer:	NIMH IRP

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

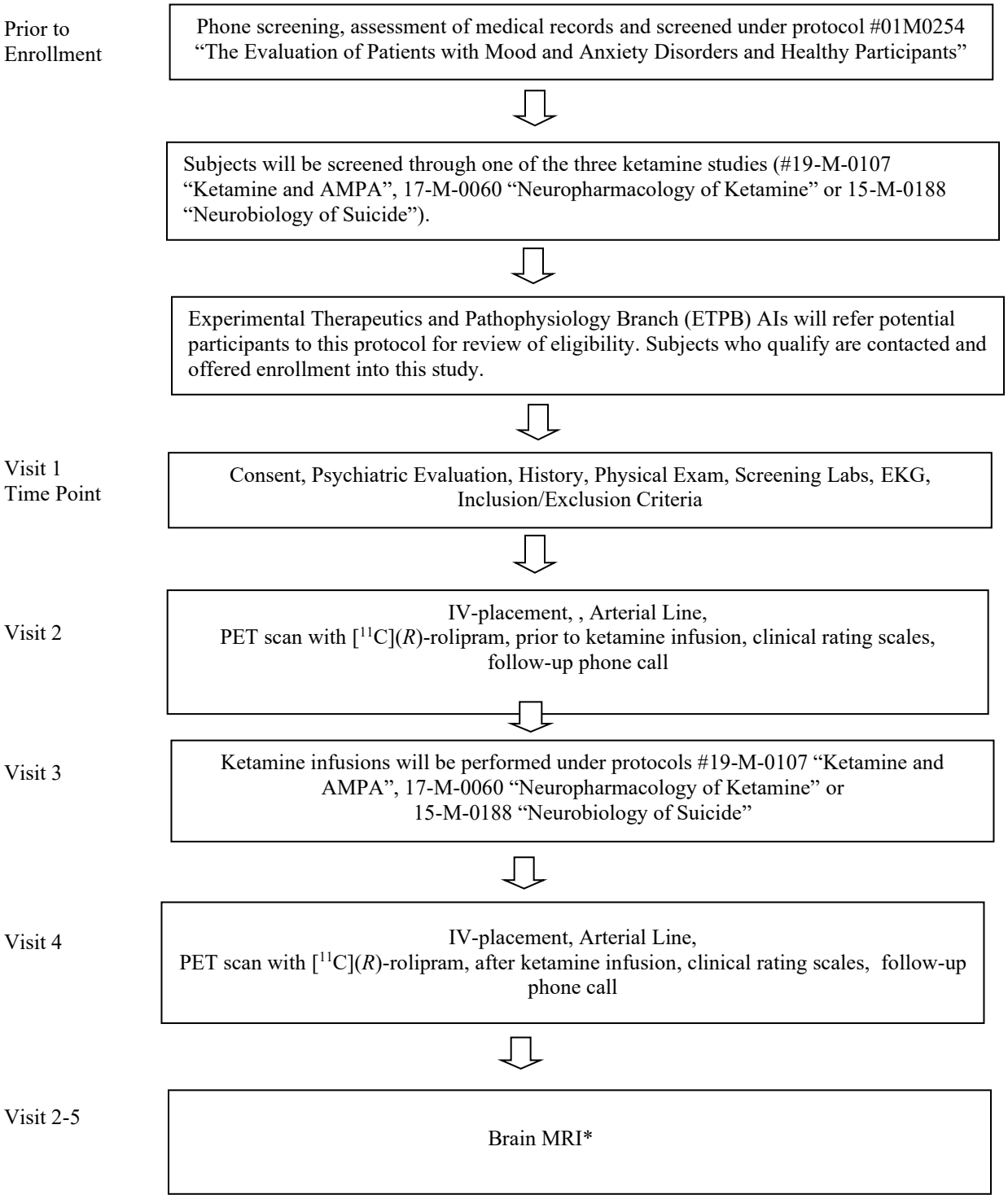
The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; an IRB determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 PROTOCOL SUMMARY

1.1 Synopsis

Title:	[^{11}C]-(R)-Rolipram to Measure cAMP Signaling Before and After Ketamine
Study Description:	This study will test the effects of ketamine infusion on the cAMP system in human brain to determine if ketamine mediates its antidepressant effects at least in part due to modulation of cAMP signaling.
Objectives:	Primary Objective: To determine if ketamine infusions in depression causes increases in cAMP signaling as measured by [^{11}C](*R*)-rolipram binding. Secondary Objectives: To determine if increases in [^{11}C](*R*)-rolipram correlate with symptomatic improvement in major depressive symptoms.
Endpoints:	Primary Endpoint: measurement of PDE4 density (volume of distribution V_T) in brains of individuals with major depressive disorder (MDD) before and after administration of ketamine. Secondary Endpoints: Clinical rating scales of depression, including MADRS, HAM-D.
Study Population:	35 inpatient participants with major depressive disorder eligible for ketamine administration and enrolled in protocols 19-M-0107 "Ketamine and AMPA", 17-M-0060 "Neuropharmacology of Ketamine" or 15-M-0188 "Neurobiology of Suicide.". Participants may be male or female. They must be between 18 and 70 years old. MDD participants must be in good medical health and provide informed consent.
Phase:	N/A
Description of Sites/Facilities Enrolling Participants:	Screening, PET imaging and ketamine infusion will be performed at the NIH Clinical Center.
Description of Study Intervention:	Participants will be intravenously injected with about 20 mCi of [^{11}C](*R*)-rolipram for 2 PET scans, prior to and after ketamine infusion. All participants will have radial artery catheters inserted for each scan to measure radioligand concentration, as well as a brain MRI to provide anatomical orientation for PET studies.
Study Duration:	36 months
Participant Duration:	Approximately six weeks depending on participant's availability and investigators' overall access to MRI and PET cameras.

1.2 Schema



* One brain MRI will be performed per participant and will be scheduled either on any visit that is logistically appropriate or on the 5th visit if it's the last thing needed.

1.3 Schedule of Activities (SOA)

The study will require one to five visits: 1) Initial evaluation, 2) Pre-ketamine PET scan, 3) ketamine infusions, 4) post-ketamine PET scan, and 5) brain MRI.

The screening evaluations during the initial visit will take 4-6 hours and will typically be performed in one day. However, these screening evaluations may be performed on different days depending on the preference of the participant and staff availability. Study procedures may take up to 6 weeks, depending on availability of PET scans and schedule of events of other protocols they may be included on. During the first visit, the participant will give informed consent and complete the screening, which includes a history and physical exam, as well as a psychiatric evaluation. The screening evaluation will include screening labs, EKG, clinical brain MRI (approx. one hour) and will typically be done on the same day. The baseline PET scan will occur prior to the initial inpatient ketamine infusion. The two PET scans will occur within an interval <6 weeks, with the post-ketamine infusion scan to occur within 1 week of ketamine infusion.

	1st Visit	**2nd Visit**	**3rd Visit**	**4th Visit**	**5th Visit**
Informed consent and Psychiatric evaluation	X				
History and physical exam	X				
Screening labs*	X	X			
Clinical rating scales		X		X	
Research blood draw	X				
EKG	X				
Ketamine Infusion(s)**			X		
PET Scan		X		X	
Brain MRI		On one of visits 2-5			

**The screening labs are described in Section 5.2, exclusion criterion #1.*

***Ketamine infusion will be performed under protocol* #19-M-0107, 17-M-0060 or 15-M-0188.

2 INTRODUCTIONStudy Rationale

Major depressive disorder (MDD)

(MDD) is a prevalent psychiatric disorder with a lifetime prevalence of 20.6% in the United States [4]. It is a major cause of disability worldwide, with suicide being the 10th leading cause of death in the United States, the second leading cause of death between the ages of 10 and 34, and the fourth leading cause of death between the ages of 35 and 44, according to CDC data in 2019. Despite decades of rigorous study, no consensus on etiology or molecular pathophysiology of MDD exists. Current treatment modalities for MDD include pharmacological treatment with antidepressant medications, most of which act to target monoamine levels in the synaptic cleft. This increase in monoamines has been shown to occur within hours to days; however, symptomatic relief, if any is noted, does not usually manifest until days to weeks after compliant medication use. Given the relatively low rates of response and remission of monoamine-targeted therapies, as well as discordance between molecular effects and symptomatic improvement, alternative frameworks for the physiological underpinnings of MDD are needed. One such framework is called the "cAMP theory of depression", which posits that low cAMP signaling predisposes to MDD, and hypothesizes that at least a subgroup of patients with MDD present with symptoms due to low cAMP signaling. Targeting the increase of cAMP is a major direction in the development of new antidepressants, among which ketamine and its metabolites have emerged as effective, rapid-onset treatments for MDD.

In addition to their effects on monoamine levels, chronic (but not acute) antidepressant use has been shown to increase cAMP production ([5, 6], Figure 1). In contrast to the delayed effectiveness of other antidepressant drugs, **Ketamine** produces rapid and robust antidepressant effects in depressed patients within hours of administration. Classically known as a noncompetitive N-methyl-D-aspartate receptor (NMDAR) antagonist, ketamine and its metabolites have been shown to increase cAMP signaling rapidly in glial cell culture models ([1]), concordant with the observed time-frame of its antidepressant action. We have previously shown a global decrease in cAMP signaling in MDD as measured by [^{11}C](*R*)-rolipram ([7]), and further that two-month treatment with antidepressants increased [^{11}C](*R*)-rolipram binding compared to baseline ([6]). We *hypothesize* that ketamine produces rapid antidepressant effects

at least in part via increases in cAMP signaling, and propose to test this hypothesis via [^{11}C](*R*)-rolipram binding before and after ketamine treatment.

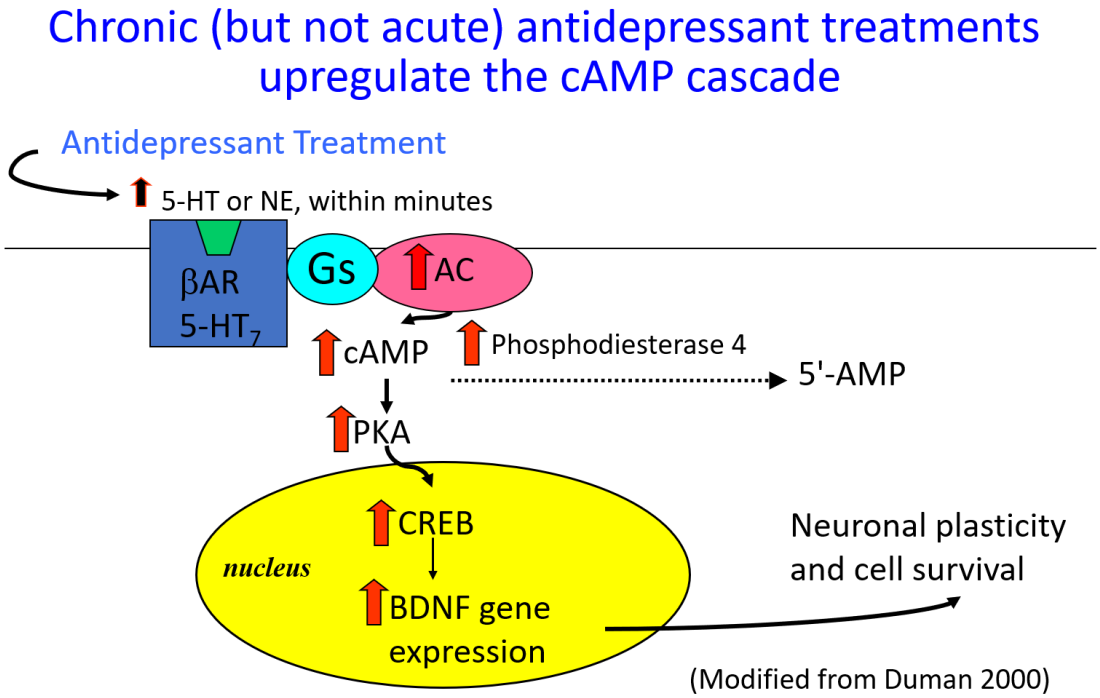


Figure 1. Chronic (but not acute) antidepressant treatments upregulated the cAMP cascade. Although serotonin (5HT) and norepinephrine (NE) are immediately increased by AD treatment (black arrows), the therapeutic response takes weeks or months. However, portions of the cAMP signaling, marked with the red arrows, are upregulated over weeks or months, as well, and may mediate the therapeutic response. In turn, cAMP upregulates both Phosphodiesterase 4 & PKA – increasing production of both 5'-AMP & CREB. Within the nucleus, CREB, a responsive transcription factor, upregulates BDNF gene expression which then promotes neuronal plasticity & cell survival, and is thought to mediate antidepressant effects. (Modified from [3])

2.2 Background

Imaging of cAMP: Studies with rolipram

PDE4 metabolizes and thereby terminates the actions of the second messenger cAMP. (**Figure 1**). Rolipram is a reversible PDE4 inhibitor, and we believe that binding of [^{11}C](*R*)-rolipram is positively correlated with cAMP signaling. Please note that this interpretation is opposite to what most would initially think, which is that increased PDE4 activity would metabolize cAMP and therefore be associated with decreased cAMP signaling. A series of rat studies (reviewed below) confirmed a negative feedback system mediated by protein kinase A (PKA). Within this system, cAMP stimulates PKA, which phosphorylates PDE4, which increases enzyme activity about 10-fold and increases the affinity of rolipram for PDE4 by about 10-fold. Therefore, the *in vivo* binding of [^{11}C](*R*)-rolipram largely reflects the activated (phosphorylated) form of PDE4, which is mediated by cAMP and PKA (**Figure 2**).

We found that [^{11}C](*R*)-rolipram binding was globally decreased in unmedicated MDD subjects experiencing a major depressive episode compared to controls [7]}. This result is consistent with the 'cAMP theory of depression', which posits that low cAMP signaling causes at least some symptoms of depression. As a follow-up, we found that, in MDD subjects, two months of treatment with an SSRI increased (i.e., normalized) [^{11}C](*R*)-rolipram binding compared to pretreatment values [6]. This result is consistent with the 'cAMP theory of the mechanism of antidepressant action'—namely, that chronic, but not acute, treatment with conventional antidepressants increases the cAMP signaling cascade, including PDE4. PDE4 is an excellent target in this regard, as the cAMP theory of depression suggests that inhibiting this enzyme should have antidepressant effects. In fact, Pfizer tested rolipram as an antidepressant in the early 1990s. The results were inconclusive, but some evidence of rapid antidepressant effects was noted. However, development of this agent was stopped early because of nausea and vomiting, possibly associated with PDE4D inhibition. Interestingly, ketamine has also been associated with nausea, although it is generally well-tolerated.

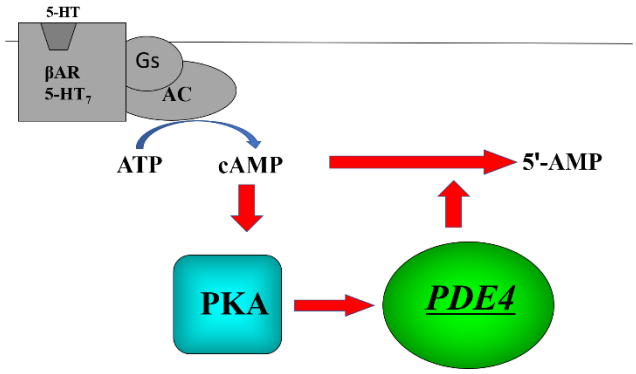


Figure 2. cAMP activates protein kinase A (PKA), which phosphorylates and activates the enzyme phosphodiesterase4 (PDE4) and increases affinity of radioligand binding of [^{11}C](*R*)-rolipram by about 10-fold. This process confirms a negative feedback loop between cAMP and PDE4.

In Vivo Density, Affinity, and Phosphorylation Status of PDE4. Our initial experiments sought to measure both the in vivo and in vitro binding site density and radioligand affinity of [^{11}C](*R*)-rolipram in rat brain [8]. We found that in vivo affinity was five-fold greater than in vitro affinity, although density was similar in both conditions. The finding was consistent with the rapid dephosphorylation of PDE4 after anoxia and death. Thus, our results implied that if we wished to measure PDE4 in its activated/phosphorylated form, we could not use postmortem tissue—such studies had to be performed in living subjects.

Our next study [9]} examined the effects of PKA modulators in conscious rats on [^{11}C](*R*)-rolipram binding compared to the much less active enantiomer [^{11}C](*S*)-rolipram. Two drugs were studied. In the first study, the cAMP analogue db-cAMP was used to activate PDE4, thus increasing activity of the cAMP-dependent PKA. PKA then phosphorylated the PDE4 enzyme and increased PDE4 activity. We also studied Rp-cAMP, a PKA inhibitor that directly inhibited PKA function, thereby decreasing PDE4 activity. Unilateral injection of the PKA activator (db-cAMP) and the PKA inhibitor (Rp-cAMP) into the striatum significantly increased and decreased [^{11}C](*R*)-rolipram binding, respectively. These effects were not caused by changes in blood flow or delivery of radioligand to brain because these agents did not affect [^{11}C](*S*)-rolipram binding. The results demonstrated that [^{11}C](*R*)-rolipram binding reflected the activated phosphorylated form of PDE4 and, more generally, supported the interpretation that increased [^{11}C](*R*)-rolipram binding was associated with increased cAMP signaling.

PDE4 Binding in MDD. Based on these preclinical findings, we hypothesized that [^{11}C](*R*)-rolipram PET in humans would provide a unique in vivo measure of PDE4 density and affinity not observable in postmortem tissue. Expanding our work, we sought to quantify the binding of [^{11}C](*R*)-rolipram as an indirect measure of this enzyme's activity in the brain of individuals with MDD compared with healthy controls [7]. This work was particularly important because animal studies had suggested that upregulation of the cAMP cascade, including PDE4, was a mechanism of action common to several antidepressant treatments (reviewed in [10]). To avoid the misleading results that can be obtained from small sample sizes, we scanned a total of 44 unmedicated, moderately depressed patients with MDD and 35 age- and gender-matched healthy controls, which is about twice the size of most PET studies in psychiatry. Notably, about half the patients were treatment-naïve. [^{11}C](*R*)-rolipram binding in brain was measured using arterial [^{11}C](*R*)-rolipram levels to correct for the influence of cerebral blood flow. MDD subjects showed a widespread, 18% reduction in [^{11}C](*R*)-rolipram binding (P=0.0001) that was not caused by different gray matter volumes. Decreased [^{11}C](*R*)-rolipram binding of a similar magnitude was observed in most brain areas. These results were the first to demonstrate that brain levels of PDE4, a critical enzyme that regulates cAMP, are decreased in unmedicated individuals with MDD *in vivo*. Furthermore, the results are in line with human postmortem and rodent studies demonstrating downregulation of the cAMP cascade in MDD, and support the hypothesis that PDE4 inhibitors—which increase cAMP cascade activity—may have antidepressant effects, as demonstrated in animal experiments.

Effect of Chronic Antidepressants on PDE4 Binding. Building on this work, and in collaboration with Dr. Carlos Zarate (NIMH), we sought to determine whether antidepressant treatment upregulates PDE4 in humans as it does in animals. Of the 44 unmedicated MDD patients described above, 23 had a follow-up [^{11}C](*R*)-rolipram scan after approximately eight weeks of treatment with an SSRI [6], which significantly increased [^{11}C](*R*)-rolipram binding (12%, p<0.001), with significantly greater increases observed in older patients (p<0.0001), although rolipram binding did not correlate with severity of baseline symptoms, and increased [^{11}C](*R*)-rolipram binding during treatment did not correlate with symptom improvement. Taken

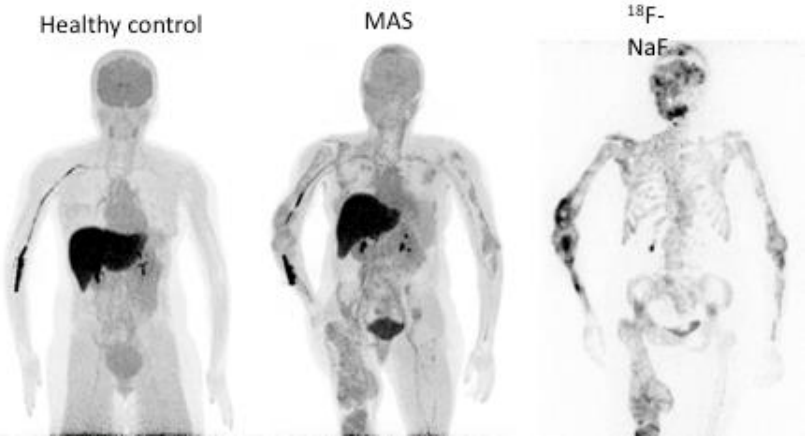


Fig. 3. [^{11}C](R)-rolipram signal coincides [^{18}F]NaF in McCune Albright Syndrome, demonstrating ability of rolipram to measure cAMP signaling. Maximum intensity projected images of [^{11}C](*R*)-rolipram of a healthy control and of a patient with MAS, and [^{18}F]NaF bone scan from the same patient. The MAS patient shows increased [^{11}C](R)-rolipram binding compared to the healthy control, which coincides with areas of fibrous dysplasia as shown on the [18F]NaF bone scan. [2]

together, these results elucidate two important and related points. First, the cAMP cascade—as indirectly measured with PDE4 binding—was downregulated in unmedicated patients with MDD. Second, antidepressant treatment normalized this [^{11}C](*R*)-rolipram downregulation. The lack of correlation between PDE4 binding and depressive symptoms could reflect the heterogeneity of the disease and/or the heterogeneity of the target, given that PDE4 has four subtypes. Nevertheless, these results suggest that PDE4 inhibition, perhaps via subtype-selective agents, might again be assessed for efficacy in MDD; the results also broadly support the cAMP theory of depression and of antidepressant action. Our goal in this project is to build on this work to determine if ketamine also targets cAMP signaling similar to SSRI treatment, and if these results correlate to clinical improvements.

Imaging PDE4 with [^{11}C](R)-rolipram in McCune Albright Syndrome Patients. Given the less-well-known connection between MDD and cAMP signaling, we decided to confirm our method of testing of [^{11}C](*R*)-rolipram to image PDE4 in McCune Albright Syndrome (MAS), a syndrome characterized by mosaicism arising from mutations in the *GNAS* gene, which activates adenylyl cyclase, and directly generating cAMP and causing fibrous dysplasia which can be measured by [^{18}F]NaF [2]. Building on our previous work that developed and tested the ability of [^{11}C](*R*)-rolipram to image PDE4, we used [^{11}C](*R*)-rolipram to measure PDE4 binding in patients with MAS and performed both brain and whole-body scans in patients with MAS and healthy controls. The whole-body scans compared uptake between areas of fibrous dysplasia and unaffected bones. [^{11}C](*R*)-rolipram uptake in peripheral organs was also compared between subjects with MAS and healthy controls. The latter comparison was important because, in MAS, some tissues may contain *GNAS* mutations even in the absence of obvious structural changes.

Brain scans with [^{11}C](*R*)-rolipram and full arterial sampling were performed on 7 healthy controls and 3 individuals with MAS. [^{11}C](*R*)-rolipram binding was increased in dysplastic bone but not in other peripheral organs, as shown by whole-body scans in individuals with MAS compared to healthy controls (Fig. 1) Areas of skeletal uptake seen on the PDE4 whole-body images coincided with areas of uptake seen in images from [^{18}F]NaF PET/CT scans. In addition, [^{11}C](*R*)-rolipram binding was not increased in the brains of individuals with MAS compared with healthy controls, and mosaicism (i.e., small areas of increased binding) was not apparent on visible inspection of the images. These results are the first confirmation in humans that increased PDE4 binding is associated with increased cAMP signaling, consistent with our interpretation of animal studies of [^{11}C](*R*)-rolipram. In addition, this study supported the utility of such PET scans to indirectly measure altered cAMP signaling in other human diseases, including major depressive disorder, if the abnormality extends to adequately sized regions of the target organ.

Evidence that ketamine mediates its antidepressant effects in part via the cAMP system

As stated previously, ketamine has been demonstrated to have rapid antidepressant effects that contrast with monoamine-associated therapies such as SSRIs. While classically ketamine is considered an NMDAR-antagonist, and has some known associations with glutamatergic signaling, there is also evidence that ketamine's antidepressant effect may be independent of NMDAR ([1],[11]). First, other NMDAR antagonists, such as memantine, have failed to consistently demonstrate the rapid and robust antidepressant effect of ketamine, despite ostensibly having a similar mechanism of action ([12]). Further, while generally ketamine treatments include a racemic mixture of both (*R*)- and (*S*)-ketamine (arketamine and esketamine

hereafter), there is a significant difference between affinities of these enantiomers for the NMDAR, with arketamine demonstrating a Ki of 1.4 uM, compared to esketamine at Ki of 0.3 uM. While esketamine is now Food and Drug Administration (FDA) approved for the treatment of depression, arketamine has been demonstrated to have a greater potency for antidepressant effects in rodent models compared to esketamine (reviewed in [11]).

Ketamine has been demonstrated to increase proliferation in neuronal progenitor cells in an NMDA-independent manner, while also increasing cAMP signaling within 15 minutes ([13]). Wray et al [1] also demonstrated that treatment of C6 glioma cells with ketamine led to increased cAMP accumulation, as well as subsequent pCREB and BDNF (Figure 4). Phosphorylated CREB (pCREB) translocates to the nucleus where it acts as a transcription factor for genes involved in growth and survival, neuroprotection, and synaptic plasticity, perhaps in part due to downstream effects of BDNF transcription [14],[15]. CREB is both upregulated and phosphorylated at Ser-133 after chronic antidepressant treatment in animals and cultured cells [15]. This effect was noted to be mediated by a ketamine-induced association between $G_{\alpha s}$ with adenylyl cyclase, a mechanism shared by prolonged antidepressant treatment[16].

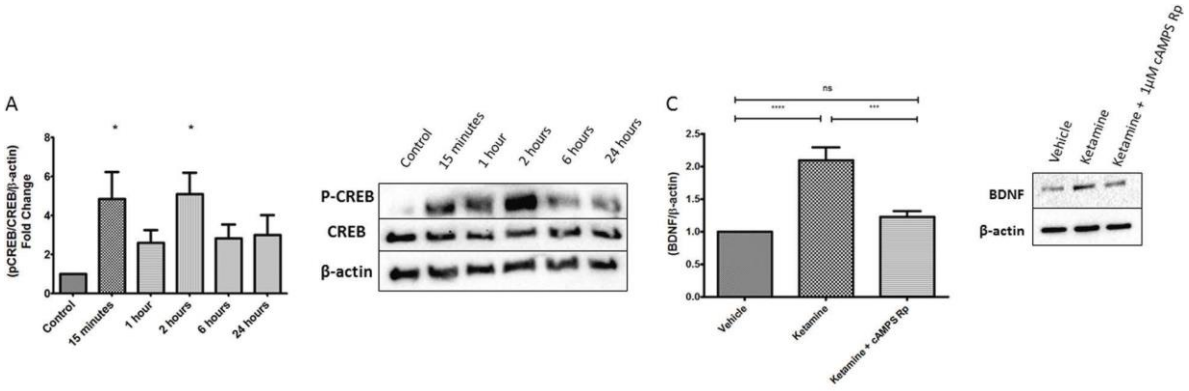


Figure 4. Ketamine treatment increases BDNF in a CREB- and cAMP-dependent manner. C9 cells treated with ketamine demonstrate increase levels of active CREB (P-CREB) in response to acute ketamine infusion, which results in downstream increases in BDNF production, an effect that is disrupted by Rp-CAMPS, an inhibitor protein kinases downstream of cAMP signaling [1].

Given a proposed mechanism demonstrated in cell culture models, as well as the clinically relevant use of ketamine in treatment-resistant patients with MDD, our proposed study will correlate changes in the cAMP signaling system in human patients treated with ketamine to determine 1) if decreased [^{11}C](*R*)-rolipram binding is corrected acutely by ketamine treatment, and 2) if any such correction will be correlated to clinical response.

2.3 Risk/Benefit Assessment

2.3.1 Known Potential Risks

Risks include those associated with: a) clinical rating scales and psychiatric assessment; b) MRI; c) EKG, d) placement of venous and arterial lines; e) arterial blood sampling; f) radiation exposure from the radioligand and from the transmission scan; g) PET scanning.

2.3.1.1 Risks of Clinical Rating Scales and Psychiatric Assessment

No discomfort is expected to occur during clinical interviews or psychological assessments, other than the potential emotional stress caused by discussing difficult psychiatric topics.

2.3.1.2 MRI

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

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

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

2.3.1.3 EKG

The patient may feel uncomfortable while the electrodes are attached to the chest. The conductive gel sometimes causes some mild irritation.

2.3.1.4 Placement of Venous and/or Arterial Line

Venous catheter insertion can be associated with discomfort, bruising, infection, or clot formation. Using proper placement techniques will minimize these risks. In case of tracer extravasation, we will stop the study, remove the venous line from the arm, and apply cold to the site. Arterial catheterization is a generally safe and reliable method of obtaining arterial blood samples [28, 29]. Placement of a radial arterial catheter may cause bruising or infection. There is also a risk of occlusion and microemboli. Over 3,000 arterial catheters have been placed to date in the PET Department. Of these, only two complications requiring physician's care arose. In the first case, a small radial artery aneurysm developed several months later, which was successfully

repaired surgically. In the second case, a radial artery thrombosis developed 28 days later, which was also successfully repaired surgically.

2.3.1.5 Blood Sampling

Participants may have arterial or venous blood sampling. The total amount of blood drawn shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any eight-week period. Blood sampling may lead to the formation of small subcutaneous hematomas caused by blood leaking from a punctured blood vessel. Such hematomas cause only minor discomfort. They are not dangerous and require no treatment other than reassuring the participant. There is also a small risk of infection at the site of the needle puncture, which can be readily treated with antibiotic therapy. We will ask participants not to donate blood within eight weeks before the study and for eight weeks following the study.

2.3.1.6 Radiation Exposure Risks

Radiation exposure in this protocol will be from [^{11}C](*R*)-rolipram and the associated transmission scans.

The radiation exposure (rem) from two injections of [^{11}C](*R*)-rolipram, 20 mCi each, which is a typical dose for brain imaging, is: effective dose (0.8). Gallbladder (1.6), lungs (2.2), and liver (1.9) are the three organs with the highest levels [30].

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

We routinely include the dose from two transmission scans for each PET scan in the event that it must be repeated in any given participant. Therefore, four CT scans might be necessary. The effective dose for four head transmission scans from a PET/CT is ~ 0.08 rem. Thus, the maximum total effective dose in each [^{11}C](*R*)-rolipram PET scan is 0.44 rem, and for the whole study it would be 0.88 rem. These estimated annual effective doses are well below the limit of 5 rem per year established by the NIH's Radiation Safety Committee. All participants will be asked about any prior research participation involving radiation exposure so that the total exposure, in combination with the present study, will not exceed an effective dose of 5 rem per year.

2.3.1.7 PET Scan

PET scans, which detect injected radioactivity within the body, are not associated with any known physical hazards to the participant lying on the table. We routinely use a series of procedures to minimize the risk of discomfort during scanning sessions. The procedures are conducted in the presence of trained health professionals to whom participants will have ready access should they experience any problems. Participants can communicate with the trained health professionals while in the scanner and can be removed from the scanner and withdraw from the study at any time if they wish to do so. Participants can also request that the operator stop the scan.

2.3.2 Known Potential Benefits

This study offers no direct benefits to the participants related to the PET scans but will likely provide fundamental knowledge regarding the mechanism of action of ketamine in the treatment of depression.

2.3.3 Assessment of Potential Risks and Benefits

If the results are positive, PET imaging with [^{11}C](*R*)-rolipram may be used to explore the pathophysiological role of cAMP in depression as well as shed light on the mechanism of action of ketamine. This valuable information may then help guide future trials with anti-depressant drugs, a benefit to future patients that justifies the risks to the current participants.

With regard to how the study design sought to minimize risks, please see each risk listed above (Section 2.3).

3 OBJECTIVES AND ENDPOINTS

Objectives	Endpoints	Justification for Endpoints
Primary		
Measurement of cAMP signaling via [^{11}C](*R*)-rolipram density (distribution volume V_T) in the brains of individuals with MDD before and after ketamine infusion	Distribution volume V_T of [^{11}C](*R*)-rolipram pre- and post-ketamine scan	Change in V_T of [^{11}C](*R*)-rolipram is a surrogate marker for cAMP signaling, a proposed mechanism for ketamine's mechanism of action
Secondary/Exploratory		
1) The relationship between clinical rating scales and PDE4B binding	1) Distribution volume V_T 2) Clinical rating scales	Correlation between V_T and clinical ratings

4 STUDY DESIGN

4.1 Overall Design

- **Site**: This single-site study will be conducted at the NIH Clinical Center.
- **Hypothesis**: We hypothesize that PDE4 levels as quantified by [^{11}C](*R*)-rolipram in the brains of subjects suffering from a major depressive episode will be increased with ketamine administration, and that these levels will correlate with clinical rating scales.
- **Type of Design**: MDD participants will be studied with [^{11}C](*R*)-rolipram prior to and after ketamine infusion.

- **Study Groups**: Individuals with MDD
- **Study intervention**: Two PET scans with [^{11}C](*R*)-rolipram and MRI of brain.
- **Interim Analysis**: None.
- **Stratifications**: None.

4.2 Scientific Rationale for Study Design

This study is exploratory and seeks to gather information that would be useful for designing a larger follow-up study.

4.3 Justification for Dose

Previous studies with [^{11}C](*R*)-rolipram used a dose of 20 mCi, which is enough to measure both radioactivity in brain with PET and the concentration of parent radioligand in arterial plasma.

5 STUDY POPULATION

5.1 Inclusion Criteria

Patients: In order to be eligible for this study, MDD participants must meet all of the following criteria:

1. Aged 18 to 70 years old.
2. Female participants of childbearing potential must be using a medically acceptable means of contraception.
3. Participants must be in good general health as evidenced by medical history and physical examination.
4. Each participant must have a level of understanding sufficient to agree to all required tests and examinations and sign an informed consent document.
5. All participants must have undergone a screening assessment under protocol 01-M-0254, "The Evaluation of Patients with Mood and Anxiety Disorders and Healthy Participants".
6. Participants must be enrolled in the ketamine arms of protocols 19-M-0107 "Ketamine and AMPA", 17-M-0060 "Neuropharmacology of Ketamine", or 15-M-0188 "Neurobiology of Suicide".
7. Participants must fulfill DSM-5 criteria for major depression (MDD) without psychotic features, as based on clinical assessment and structured diagnostic interview (SCID-P).
8. Participants must have an initial score on the MADRS ≥ 18 or HAM-D ≥ 15 within two weeks of study entry.
9. Participants with stable medical conditions as assessed by their primary care provider (PCP) and/or in-house clinician are permitted to join the study.
10. Patients must qualify for ketamine administration, usually defined as lack of response to two adequate lifetime antidepressant trials, with [at least] one in the current major depressive episode, operationally defined using the Antidepressant Treatment History Form (ATHF); a failed adequate trial of ECT [or TMS] would count as an adequate antidepressant trial.
11. Participants must have their radial artery pulse checked for the presence of adequate ulnar collateral flow and the absence of any metal or foreign objects in both wrists.
12. Participants must agree to adhere to the lifestyle considerations (see Section 5.4).

5.2 Exclusion Criteria

Participants with MDD who meet any of the following criteria will be excluded from participation in this study:

1. Clinically significant abnormalities on EKG or laboratory testing. This includes CBC, acute care panel (Na, K, Cl, CO_2, creatinine, glucose, urea nitrogen); prothrombin and partial prothrombin tests, the PT/PTT is optional for those without history of coagulation disorders or those not on anticoagulation.
2. Current psychotic features, a diagnosis of schizophrenia or any other psychotic disorder as defined in the DSM-5.
3. Participants with a history of DSM-5 substance use disorder (except for caffeine or nicotine dependence) within the preceding three months. In addition, participants must not have substance use disorder or alcohol use disorder. However, alcohol or cannabis use by themselves are not exclusion criteria, unless that use affects the function of daily life.
4. Participants who, in the investigator's judgment, pose a current serious suicidal or homicidal risk.
5. Participants who have a history of aggressive behavior towards others.
6. Participants who have an unstable medical condition that, in the opinion of the investigators, makes participation unsafe (e.g., an active infection or untreated malignancy).
7. Are unable to travel to the NIH.
8. Have recent exposure to radiation related to research (e.g., PET from other research) that, when combined with this study, would be above the allowable limits.
9. Have an inability to lie flat and/or lie still on the camera bed for at least two hours, including claustrophobia, overweight greater than the maximum for the scanner, and uncontrollable behavioral symptoms, which will be screened by an interview with the participant during the screening visit.
10. Are unable to have an MRI scan (e.g., because of pacemakers or other implanted electrical devices, brain stimulators, dental implants, aneurysm clips (metal clips on the wall of a large artery), metallic prostheses (including metal pins and rods, heart valves, and cochlear implants), permanent eyeliner, implanted delivery pumps, shrapnel fragments, or metal fragments in the eye.
11. Be NIMH staff or an NIH employee who is a subordinate/relative/co-worker of the investigators.
12. Pregnancy
13. HIV infection

5.2.1 Exclusion of Children

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

5.2.2 Exclusion of Pregnant or Breastfeeding Women

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

5.2.3 Exclusion of Participants who are HIV Positive

Persons with HIV infection are excluded because HIV infection itself may change cAMP signaling.

5.3 Inclusion of Vulnerable Participants

5.3.1 Safeguards for Vulnerable Populations and Sensitive Procedures

5.3.1.1 Role of the HSPU

The Human Subjects Protection Unit will monitor the consent process for all MDD participants.

5.3.2 Participation of NIH Staff or family members of study team members

NIH staff may be enrolled in this study as the population meets the study entry criteria and NIH Policy 404 will be followed. There will be no direct solicitation of NIH employees, nor will they be directly recruited by or through their supervisors or co-workers to participate in this study. Neither participation nor refusal to participate as a subject in the research will have an effect, either beneficial or adverse, on the participant's employment or position at NIH. NIH employees must have permission from their supervisor and/or take leave in order to receive compensation if participation is during work hours. Prior to enrollment, the NIH employee will be requested to review the Annual Leave Policy for NIH Employees. Participant confidentiality and privacy will strictly be held in trust by the participating investigators, their staff, and the sponsor(s).

Per NIMH Policy, NIMH employees, staff and their immediate family members will be excluded from the study due to ethical concerns about confidentiality and conflict of interest. A copy of the NIH Frequently Asked Questions (FAQs) for Staff Who are Considering Participation in NIH Research) will be made available for NIH employees who wish to participate in this study.

This study collects sensitive medical information. Every effort will be made to protect participant information, but such information may be available in medical records and may be available to authorized users outside of the study team in both an identifiable an unidentifiable manner. The PI will train study staff regarding obtaining and handling potentially sensitive and private information about co-workers through staff discussions.

5.4 Lifestyle Considerations

During this study, participants will be asked to abstain from strenuous exercise and from lifting more than 10 pounds using the affected arm for 48 hours after removal of the arterial line. Patients will be asked not to donate blood for 8 weeks before or 8 weeks after PET scans.

5.5 Screen Failures

Screen failures are defined as participants who consent/assent to participate in the clinical trial but are not subsequently assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements, and to respond to queries from regulatory authorities. Minimal information includes demographic characteristics, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this trial (screen failure) because of an exclusion criterion that may resolve by the time of treatment may be rescreened. Rescreened participants should be assigned the same participant number as for the initial screening.

5.6 Strategies for Recruitment and Retention

Participants will be recruited from protocol #'s 19-M-0107 "Ketamine and AMPA", 17-M-0060 "Neuropharmacology of Ketamine" or 15-M-0188 "Neurobiology of Suicide."

The protocol and contact information to obtain further details will be listed on www.clinicaltrials.gov.

5.6.1 Costs

We expect participants to incur no costs for participating in this study.

5.6.2 Compensation

Participants will be compensated for time- and research-related inconveniences. Reimbursement is based on NIH standards for time devoted to the research project. Participants will be paid for each portion of the study they complete whether or not they opt for early withdrawal from participation. Without any delay of study procedures or unanticipated inconvenience, the total possible compensation for procedures is $730. Participants will also receive compensation for time spent at each visit following the structure of $20 for the first hour and $10 for each subsequent hour. If the investigators need to delay study procedures or if additional time is need for completion, participants may receive additional compensation in accordance with NIH guidelines.

Participants may receive support for travel, meals, and lodging according to NIH travel policy guidelines. Lodging may be provided directly or reimbursed according to NIH guidelines. For NIH employees, guidelines per Policy 404 will be followed. Employees and staff who participate during work hours must have permission from their supervisor. NIH employees must either participate outside of work hours or take leave in order to receive compensation.

Visit 1 to NIH	
Screening	$50
Pregnancy test	$10
Psychiatric evaluation	$50
Visit 2to NIH	

PET scanning	$150
Arterial catheter	$60
Antecubital venous catheters	$30
Pregnancy test	$10
Movement restriction	$10
*Visit to NIH 3 **	N/A
Visit 4 to NIH	
PET scanning	$150
Arterial catheter	$60
Antecubital venous catheters	$30
Pregnancy test	$10
Movement restriction	$10
Visit 5 to NIH	
MRI	$100
Total	$730

**Ketamine infusions will be performed under protocols #19-M-0107 "Ketamine and AMPA", 17-M-0060 "Neuropharmacology of Ketamine" or 15-M-0188 "Neurobiology of Suicide*

6 STUDY INTERVENTION

6.1 Study Interventions(s) Administration

6.1.1 Study Intervention Description

Each participant will receive two injections of [^{11}C](R)-rolipram, prior to and after ketamine infusion, and will also receive an MRI.

6.1.2 Dosing and Administration

Each participant will be intravenously injected with about 20 mCi of [^{11}C](*R*)-rolipram for each PET scan. The yield of the radioligand synthesis varies such that the available dose may be more or less than 20 mCi. By prior agreement with NIH's Radiation Safety Committee, the clinician covering the scan can approve a dose as low as half of the prescribed dose. We expect that a dose of about 20 mCi will likely be adequate for all participants per scan. The maximal dose will provide the best accuracy to measure radioactivity in brain with PET and the parent radioligand in plasma with radioHPLC.

6.1.2.1 Dose Escalation

None.

6.1.2.2 Dose Limiting Toxicity

Toxicity from this radiolabeled drug comes from radioactive emissions and the mass dose of the nonradioactive carrier. Both the injected radioactivity and mass dose are many-fold lower than that required to cause toxicity.

6.1.2.3 Dose Modifications

The dose will not be modified by toxicity. Instead, the dose may be less than that prescribed, based on the PI's judgment of the magnitude of noise that will be acceptable in the brain and blood measurements.

6.1.2.4 Drug Administration

The radioligand will be injected intravenously.

6.2 Preparation/Handling/Storage/Accountability

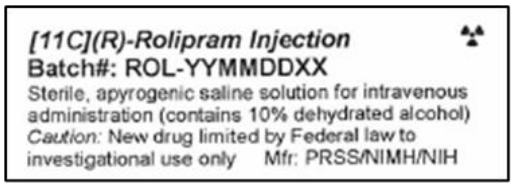
The radioligands are prepared by the NIMH radiochemistry laboratory and handled by the PET Department radiopharmacy according to the associated INDs and SOPs. The radioligands have minimal storage because they must be injected within one hour of their preparation.

6.2.1 Acquisition and Accountability

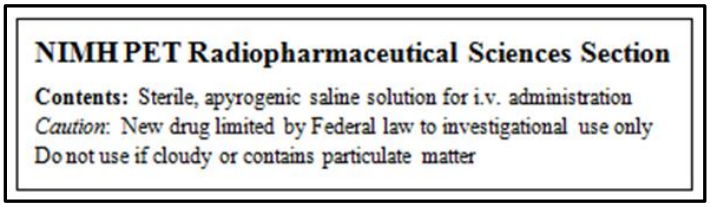
The radioligand will be delivered by the NIMH radiochemistry laboratory to the PET Department's radiopharmacy. Acquisition and accountability are the responsibility of the NIMH radiochemistry laboratory.

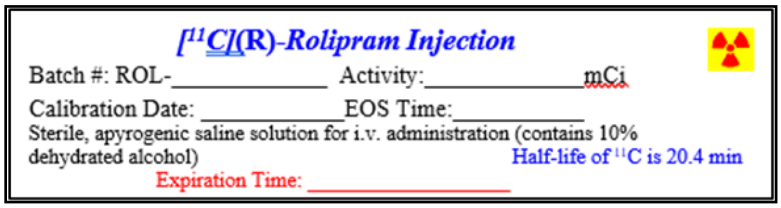
6.2.2 Formulation, Appearance, Packaging, and Labeling

Each product will be formulated in sterile, apyrogenic saline containing 10% dehydrated alcohol in a single-use vial and labeled according to requirements for PET drug products described in USP Chapter <823>. The following label will be affixed directly to the vials prior to filling:



Two additional labels are placed on the outer lead shielding used to transport the vial to the radiopharmacy:





6.2.3 Product Storage and Stability

Products are stable at controlled room temperature for the one-hour expiration period and have no additional storage requirements. Product vials are expected to be single-use, but if the seal is broken and additional product must later be withdrawn, the same storage conditions and original expiration time would apply.

6.2.4 Preparation

Products are provided as sterile, directly injectable solution in a multi-dose vial. The PET radiopharmacist will aseptically remove from the multi-dose vial only the volume required for a participant dose. If necessary, this volume will be diluted with 0.9% normal saline, USP to 12 mL

6.3 Measures to Minimize Bias: Randomization and Blinding

This study is neither randomized nor blinded.

6.4 Study Intervention Compliance

This study is neither randomized nor blinded.

6.5 Concomitant Therapy

Not applicable.

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

7.1 Discontinuation of Study Intervention

Discontinuation instituted by the investigator has been rare in our PET studies, but would likely occur during one of the two or three scans (i.e., MRI, one or two PETs). Examples include a participant experiencing such anxiety that the investigator recommends study discontinuation, even though the participant is willing to proceed. More specifically, the PI may discontinue or withdraw a participant from the study for the following reasons:

- Disease progression that requires discontinuation of the study intervention, which is unlikely for this relatively short study.
- If any clinically adverse event (AE)or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant.
- Investigator discretion – e.g., excessive anxiety of the participant
- Positive pregnancy test.

Any new clinically relevant finding will be reported as an AE, and the cause of discontinuation will be recorded in CRIS. In addition to discontinuation instituted by the PI, the participant may discontinue (i.e., withdraw) from the study at any point for any reason.

7.2 Participant Discontinuation/Withdrawal from the Study

Prior to removal from study, effort must be made to have all subjects complete a safety visit approximately 2 days following the last dose of study therapy.

Participants are free to withdraw from participation in the study at any time upon request.

An investigator may discontinue or withdraw a participant from the study for the following reasons:

- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation
- Subject has completed the study follow-up period
- Death
- Screen Failure

The reason for participant discontinuation or withdrawal from the study will be recorded on the Case Report Form (CRF).

7.3 Lost to Follow-up

A participant will be considered lost to follow-up if s/he fails to return for a scheduled visit and is unable to be contacted by the study site staff. The following actions must be taken if a participant fails to return to the clinic for a required study visit:

• The site will attempt to contact the participant and reschedule the missed visit as soon as practicable and counsel the participant on the importance of maintaining the assigned visit schedule. Concomitantly, we will ascertain whether the participant wishes to and/or should continue in the study.

• Before a participant is deemed lost to follow-up, the PI or their designee will make every effort to regain contact with the participant (where possible, three telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts will be documented in the participant's medical record or study file.

• Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 Screening Procedures

Screening activities performed prior to obtaining informed consent:

Minimal risk activities that may be performed before the subject has signed a consent include the following:

- Email, written, in person or telephone communications with prospective subjects.
- Review of existing medical records to include H&P, laboratory studies, etc.

- Review of existing MRI, x-ray, or CT images.
- Review of existing photographs or videos.
- Review of existing pathology specimens/reports from a specimen obtained for diagnostic purposes.

Screening activities performed after a consent has been signed:

Prior to consenting to this study, participants will have undergone initial screening under protocol 01M0254, which allow for the sharing of collected data. With the first visit under this protocol we will require that all participants have an updated physical examination, psychiatric evaluation and specific laboratory tests as outlined in the Schedule of Events. Results of these tests will identify participants who should be excluded because of active medical problems or make participation in the protocol unsafe.

The lab tests are described in Section 5.2 (Exclusion Criteria #1). MDD participants will be evaluated for their ability and willingness to tolerate study procedures such as arterial line and lying still in the PET scanner.

8.2 Study Evaluations & Procedures

In addition to the screening procedures described in Section 8.1, all participants will undergo:

- *Brain MRI*. A brain MRI will be obtained for anatomic localization and will be performed on a 3 Tesla scanner located at the NIH Clinical Center (Bethesda, Maryland). The MRI will take about one hour. Participants will undergo safety screening prior to the MRI to rule out contraindications such as cardiac pacemaker. If the participant has had a recent brain MRI suitable for PET imaging quantification, this test need not be repeated.
- *Pregnancy Tests.* For women of childbearing potential, urine pregnancy testing will be done within the 24 hours prior to any MRI or PET scan. If the pregnancy test is positive, PET and MRI will not be done, and the participant will be removed from the protocol.

8.2.1 PET Procedures

- *Radioligand*. [^{11}C](*R*)-rolipram will be prepared according to the IND and administered via an indwelling intravenous catheter over approximately one to three minutes.
- *Insertion of the intravenous line*. An intravenous line will be placed in the arm. The venous line will be used to inject the radioligand and will be removed at the end of the day. The IV line may be replaced if it fails to remain patent.
- *Arterial line placement*. After the presence of adequate ulnar collateral flow has been confirmed, a radial artery catheter will be inserted by the Anesthesiology Department or the Vascular Access Department. Before catheter insertion, the skin at the puncture site will be locally anesthetized.
- *PET scans*. Brain PET imaging will be performed using a PET/CT scanner. Participants will be placed on the scanner bed. The participant's head may be secured with a head holder or a thermoplastic mask to prevent excessive motion, but the mask can be removed if the participant finds it uncomfortable. A CT transmission scan will

be performed to measure and correct for attenuation. Tracer infusions will be performed when the participant is already on the scanner bed. After an intravenous bolus of each radioligand, we will collect about 25 arterial samples. More blood samples may be necessary for longer scans. Arterial sampling will initially be performed continuously at early time points and discretely at later time points, but this plan can be modified if required to improve the quality of the data. The total amount of blood withdrawal is described in Section 8.2.3. PET images will be acquired for up to four hours. The scans generally will last approximately 90 minutes each, with the initial scan prior to ketamine infusion, and the subsequent scan to be performed up to 1 week after ketamine infusion. When the scan is completed, the arterial and/or venous lines will be removed, and the participant will be instructed to void frequently to minimize radiation exposure.

8.2.2 Clinical Rating Scales

The following rating scales may be administered to MDD participants at baseline (within six weeks of entry into the protocol). Clinical rating scales also will be performed after ketamine infusion, and will be performed by MIB clinical staff, unless these scales are to be performed post-ketamine by ETPB staff under their protocols, in which case the scales will not be repeated.

Beck Depression Inventory, Second Edition (BDI-II)

The BDI-II is a 21-question, multiple-choice, self-report inventory [32]. The BDI-II is used to assess the existence and severity of symptoms of depression. In addition, responses indicate changes in sleep, appetite, agitation, concentration, and energy. When completing the BDI-II, individuals are asked to consider each statement as it relates to the way they have felt for the past two weeks. The estimated time to administer this scale is 10-15 minutes.

Hamilton Psychiatric Rating Scale for Anxiety (HAM-A)

The HAM-A [33] is a widely used observational rating measure of anxiety severity. The scale consists of 14 items. Each item is rated on a scale of 0 to 4. This scale will be administered to assess the severity of anxiety and its improvement during the course of therapy. The HAM-A total score is the sum of the 14 items and the score ranges from 0 to 56. The estimated time to administer this scale is 20 minutes.

Hamilton Depression Rating Scale (HAM-D)

The HAM-D [34] is a widely used observational rating measure of depression severity. The HAM-D will be administered to assess the severity of depressive symptoms. It assesses both the presence and severity of individual signs and symptoms characterizing depression without psychotic features. The estimated time to administer this scale is 30 minutes.

Montgomery-Asberg Depression Rating Scale (MADRS)

The MADRS [35] is a 10-item instrument used to evaluate depressive symptoms in adults and to assess any changes to those symptoms. Inter-rater reliability of the scale is high, and scores correlate significantly with those of the HAM-D. Each of the 10 items is rated on a scale of 0 to 6, with differing descriptors for each item. These individual item scores are added together to form a total score, which can range between 0 and 60 points. The estimated time to administer this scale is 20 minutes.

Massachusetts General Hospital Cognitive and Physical Functioning Questionnaire (MGH-CPFQ)

The MGH-CPFQ [36] is a brief (7-item) self-report inventory used to assess rates of significant cognitive symptoms, sleepiness, and fatigue. The estimated time to administer this scale is five minutes.

Snaith-Hamilton Pleasure Scale (SHAPS)

The SHAPS [37] is a 14-item self-report instrument developed to assess hedonic capacity and has been validated in participants with MDD. The estimated time to administer this scale is five minutes.

Clinician-Administered Dissociative States Scale (CADSS)

The CADSS is a clinician-administered measure of perceptual, behavioral and attentional changes occurring during dissociative experiences that has been tested in healthy subjects and post-traumatic stress disorder (PTSD). This scale involves 19 self-reported questions and eight observer ratings scored from 0 (not at all) to 4 (extremely). To characterize dissociative responses to ketamine, the CADSS will be sorted into five subscores with apparent face validity based on published scales that also assess dissociative states: body perception, environmental perception, feelings of unreality, memory impairment and time perception.

These rating scales include questions regarding depression and suicidality. If during the course of these evaluations, suicidality is noted, a clinical risk assessment will be performed by a licensed clinician, and the NIMH psychiatric consultation team will be consulted as needed for acute safety concerns. Otherwise, participants will be referred to their mental health provider as appropriate.

8.2.3 Biospecimen Evaluations

After injection of the radioligand, arterial sampling will be performed continuously at early time points and discretely at later time points, but this plan can be modified if required to improve quality of data. Participants may have arterial or venous blood sampling. The total amount of blood drawn shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any eight-week period.

8.2.4 Correlative Studies for Research/Pharmacokinetic Studies

The concentration of parent radioligand in arterial plasma is used in conjunction with PET measurements to calculate the density of PDE4 in brain.

8.2.5 Samples for Genetic/Genomic Analysis

No samples will be collected for genetic or genomic analyses.

8.3 Safety and Other Assessments

a. Samples for safety bloods will not be collected on the day of the PET scan. .
b. Pregnancy Tests. For women of childbearing potential, urine pregnancy testing will be done within the 24 hours prior to any MRI or PET scan. If the pregnancy test is positive, PET and MRI will not be done, and the participant will be removed from the protocol.

c. Follow-up Procedures. Participants will be contacted one to three business days after each PET scan to determine whether they have had any untoward sequelae.
d. Participants will continue to see their mental health care providers throughout their participation and monitoring for worsening depressive symptoms and suicidality.
e. Study Team will communicate with the subject's PCP mental health team to ensure that they are aware that the participant is participating in the study.

8.4 Adverse Events and Serious Adverse Events

8.4.1 Definition of Adverse Event

AEs are defined as any untoward medical occurrence associated with the use of an intervention in humans, whether or not the AE is considered intervention-related (21 CFR 312.32 (a)).

8.4.2 Definition of Serious Adverse Events (SAE)

An AE or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes: death; a life-threatening AE; inpatient hospitalization or prolongation of existing hospitalization; a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions; or a congenital anomaly/birth defect. Important medical events that do not result in death, are not life-threatening, or do not require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

8.4.3 Classification of an Adverse Event

8.4.3.1 Severity of Event

The following guidelines will be used to describe severity.

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Notably, the term "severe" does not necessarily equate to "serious".

8.4.3.2 Relationship to Study Intervention

All adverse events (AEs) must have their relationship to study intervention assessed by the investigator who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.
- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.
- **Potentially Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.
- **Unlikely to be related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).
- **Not Related** – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.]

8.4.3.3 Expectedness

The PI will be responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

8.4.4 Time Period and Frequency for Event Assessment and Follow-Up

The occurrence of an AE or SAE may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate case report form (CRF). Information to be collected includes event description, time of onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of

resolution/stabilization of the event. All AEs occurring during the study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. AEs characterized as intermittent require documentation of onset and duration of each episode.

Credentialed clinicians will record all reportable events with start dates occurring any time after informed consent is obtained until seven (for non-SAEs) or 30 days (for SAEs) after the last day of study participation. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization.

8.4.5 Adverse Event Reporting

Non-SAEs will be reported to the ISM (annually), to the IRB at the time of Continuing Review and to the Sponsor at regular intervals per request.

8.4.6 Serious Adverse Event Reporting

It is both the Principal Investigator's (PI) and the Sponsor's responsibility to ensure the safety of those on the clinical trial. The PI is responsible for tracking adverse events during the study and providing adverse events lists to the Sponsor at regular intervals per request. These may be requested quarterly and will be requested no less than once a year at the time of IND annual report to the FDA. ALL AEs that are collected, as determined by the written protocol, should be tracked in the ORSC RSS' template AE Tracker or similar document. If the sponsor determines that adverse events are occurring more frequently or more severely than the written protocol had expected and/or anticipated, this should be submitted in an IND Safety Report, as described below. In addition, the PI is responsible for updating the Sponsor about known risks from the drug, as discovered from literature searches or other means.

In accordance with the requirements of 21 CFR 312.32, the PI or designee will report all SAEs, whether or not these are considered related to the investigational drug or study intervention, that occur throughout the study to the Sponsor, including those events listed in the protocol or Investigator's Brochure as anticipated to occur, as follows:

Deaths: within 24 hours of the investigator's awareness

All other SAEs: within 48 hours of the investigator's awareness

All AEs will be sent to the Sponsor quarterly, unless requested more or less frequently, for submission to the FDA in the IND Annual Report.

*"Investigator's awareness" includes awareness by anyone on the study team.

The PI will immediately report all deaths and SAEs to the Sponsor by disclosing all event-related information in a completed MedWatch Form 3500A. This form should include the IND number, protocol number, PI name, and an assessment on the reasonable possibility of a relationship between the event and the study drug or intervention. **MRNs should NOT be**

included on this form. The completed MedWatch Form 3500A will be sent **ENCRYPTED** to the Clinical Director/CEO and/or designated medical monitor with a copy to the NIH Office of Research Support & Compliance (ORSC) Regulatory Support Section.

The Clinical Director/CEO and/or designated medical monitor will be responsible for determining whether the event is reportable to the FDA as an IND Safety Report if it is a serious, unexpected, and suspected adverse reaction (SUSAR). If the sponsor determines the SAE meets the criteria of a SUSAR, the ORSC will submit an Initial IND Safety Report to the FDA no later than 15 calendar days after the PI's notification of the event to the Sponsor. Deaths or life-threatening events will be reported to the FDA no later than 7 calendar days after the PI's notification of the event to the Sponsor. The Sponsor will submit any relevant additional information in a Follow-up IND Safety Report no later than 15 calendar days after receiving the information. All SAEs will be monitored until satisfactory resolution. All AEs and SAEs will be documented on appropriate study records.

8.4.7 NIH Intramural IRB Reporting of IND Safety Reports

Only IND Safety Reports that meet the definition of an unanticipated problem or is new information that might affect the willingness of subjects on the NIH study to enroll or remain in the study will need to be reported to the NIH Intramural IRB.

8.4.8 Events of Special Interest

None

8.4.9 Reporting of Pregnancy

Participants will be excluded if they are or may become pregnant.

8.5 Unanticipated Problems

8.5.1 Definition of Unanticipated Problems (UP)

Any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied; and
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others (which many include research staff, family members or other individuals not directly participating in the research) at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or expected.

8.5.2 Unanticipated Problem Reporting

The investigator will report unanticipated problems (UPs) to the NIH Institutional Review Board (IRB) as per Policy 801.

9 STATISTICAL CONSIDERATIONS

9.1 Statistical Hypothesis

- Primary Endpoint(s): V_T of [^{11}C](*R*)-rolipram prior to ketamine infusion compared to V_T of [^{11}C](*R*)-rolipram after ketamine infusion, within one week. We hypothesize that ketamine infusion will increase rolipram binding similar to that previously described for SSRI treatment.
- Secondary Endpoint(s): We will explore correlations between changes in [^{11}C](*R*)-rolipram binding and clinical rating scales.

9.2 Sample Size Determination

The only existing database of patients scanned for PDE4 at baseline and after treatment is the one described by Fujita et al. This work, whose patients were very similar to the ones targeted in this study, analyzed 23 MDD subjects with ^{11}C-rolipram at baseline and after 8 weeks of antidepressant SSRI treatment. MDD subjects had a ~20% reduction in rolipram binding compared to controls, and the SSRI treatment significantly increased ^{11}C-rolipram binding by 12% ($P<0.001$). Consequently, we aim at studying a population of the same size as the one that revealed a significant change with pharmacological treatment. Considering dropouts, we wish to recruit up to 35 subjects, with the goal of scanning 23. Correlation/regression analyses will be performed by controlling for possible confounding factors such as age and sex.

9.3 Populations for Analyses

Patients with major depressive disorder that qualify for ketamine treatment.

9.3.1 Evaluable for toxicity

No toxicity is expected from the radioligand.

9.3.2 Evaluable for objective response

Participants will be evaluated on the basis of completion of two PET scans as the primary endpoint. Ketamine infusion has been shown to exert an antidepressant effect, which will be evaluated based on changes in clinical rating scales from pre-ketamine baseline, which will then be correlated to change in PET scans.

9.3.3 Evaluable Non-Target Disease Response

Participants may exhibit changes in clinical rating scales, in [^{11}C](*R*)-rolipram binding, neither, or both. Correlations will be made to determine if clinical improvement based on ketamine infusion is associated with [^{11}C](*R*)-rolipram binding.

9.4 Statistical Analyses

9.4.1 General Approach

For the primary endpoint of change in [^{11}C](*R*)-rolipram binding, we will compare the pre-ketamine scans within each subject with their post-ketamine scans using paired sample *t*-test.

For the secondary endpoint “Is there a correlation between V_T and clinical rating scales?”, we will use correlation analyses between the modeling results and the results of the clinical scales.

9.4.2 Analysis of the Primary Endpoints

[^{11}C](*R*)-rolipram binding assessed as V_T will be compared within subjects with a paired sample *t*-test. V_T will be calculated using pharmacokinetic modeling.

9.4.3 Analysis of the Secondary Endpoint(s)

The outcome measures for the correlative studies will be the V_T and the results of the clinical rating scales.

9.4.4 Safety Analyses

None.

9.4.5 Baseline Descriptive Statistics

Demographic variables, such as age and weight, will be summarized as mean +/- SD.

9.4.6 Sub-Group Analyses

The sample will be analyzed by sex and age, but is too small to be analyzed by other demographic characteristics.

9.4.7 Tabulation of individual Participant Data

Individual participant data will be listed by measure and timepoint.

9.4.8 Exploratory Analyses

None.

10 REGULATORY AND OPERATIONAL CONSIDERATIONS

10.1 Informed Consent Process

10.1.1 Consent/Assent Procedures and Documentation

Only the study investigators designated to obtain consent will be allowed to do so. All study investigators obtaining informed consent must have completed the NIMH HSPU training "Elements of Successful Informed Consent". When consent is conducted for subjects with MDD, HSPU must be present.

The informed consent document will be provided as a physical or electronic document to the participant or consent designee as applicable for review prior to consenting. A designated study investigator will carefully explain the procedures and tests involved in this study, and the associated risks, discomfort and benefits. In order to minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss it with friends, family members and/or other advisors, and to ask questions of any designated study investigator. A signed informed consent document will be obtained prior to any research activities taking place.

The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved remote platforms used in compliance

with policy, including HRPP Policy 303) per discretion of the designated study investigator and with the agreement of the participant/consent designee(s). Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and participant when in person) will be located in a private area (e.g., clinic consult room). When consent is conducted remotely, the participant will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed. If the consent process is occurring remotely, participants and investigators will view individual copies of the approved consent document on screens at their respective locations; the same screen may be used when both the investigator and the participant are co-located but this is not required.

Note: When required, the witness signature will be obtained similarly as described for the investigator and participant below.

When a hand signature on an electronic document is used for the documentation of consent, this study will use the following electronic platform to obtain the required signatures:

- iMedConsent platform (which is 21 CFR Part 11 compliant)

Both the investigator and the participant will sign the electronic document using a finger, stylus or mouse. Electronic signatures (i.e., the "signature" and a timestamp are digitally generated) will not be used.

The consent process will be documented in CRIS. A copy of the consent form will be given to the participant and also uploaded in CRIS.

10.1.2 Consent for minors when they reach the age of majority

Minors will not be enrolled in the study.

10.1.3 Considerations for Consent of NIH staff, or family members of study team members

Consent for NIH employees will be obtained as described in Section 5.3. 2

10.1.4 Consent of Subjects who are, or become, decisionally impaired

Adults unable to give consent are excluded from enrolling in the protocol. For those subjects that become incapacitated, they will be removed from the study.

10.2 Study Discontinuation and Closure

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. If the study is prematurely terminated or suspended, the PI will promptly inform study participants, the IRB, and the sponsor. Study participants will be contacted, as applicable, and be informed of changes to the study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants.
- Demonstration of efficacy that would warrant stopping.
- Insufficient compliance with protocol requirements.
- Data that are not sufficiently complete and/or evaluable.
- Determination that the primary endpoint has been met.
- Determination of futility.

The study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, the IRB, and/or the Food and Drug Administration (FDA).

10.3 Confidentiality and Privacy

Participant confidentiality and privacy are strictly held in trust by the participating investigators, their staff, and the sponsor(s). This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence.

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

The study monitor, other authorized representatives of the sponsor, representatives of the IRB, and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at the NIH for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, institutional policies, or sponsor requirements.

To further protect the privacy of study participants, a Certificate of Confidentiality (COC) has been issued by the NIH. This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, COCs help achieve the research objectives and promote participation in studies by helping ensure confidentiality and privacy to participants.

See Section 10.4 for information regarding sharing of research data during or after completion of the study.

10.4 Future use of Stored Specimens and Data

Genetic testing will not be performed in this protocol. No samples will be stored or shared.

In the consent form, we ask permission of the participant (yes/no response) to share imaging and other research data during or after completion of the study with collaborating laboratories at the NIH or outside of the NIH and/or submitted to open-access repositories for secondary research that may or may not involve a collaboration with the NIMH. Such open access repositories (e.g., OpenNeuro, sponsored by the NIMH) allows anyone to access the data for any purpose. Data will be stripped of all identifiers, including name, address, contact information, and medical record number prior to sharing. In addition, the face will be removed from MRI images. The data may be coded, but the key to the code will not be provided to any collaborator or party external to the NIH. After the study is completed, the de-identified data and

the code may be indefinitely maintained at the NIH and used for secondary analyses. In contrast to data, no biological samples will be shared.

10.5 Safety Oversight

Monitoring for this study will be performed by the independent safety monitor (ISM), Kenneth Towbin, MD, who is a board-certified psychiatrist with full clinical privileges at the NIH Clinical Center.

The PI will prepare a report on data and safety parameters for the ISM approximately every 12 months. The ISM will provide a written monitoring report to be submitted to the IRB at the time of continuing review.

10.6 Clinical Monitoring

Clinical site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial complies with the currently approved protocol/amendment(s), with International Conference on Harmonization Good Clinical Practice (ICH GCP), and with applicable regulatory requirement(s).

As per ICH-GCP 5.18 and FDA 21 CFR 312.50 clinical protocols are required to be adequately monitored. Monitoring for the NIH site will be conducted according to the "NIMH Intramural Program Guidelines for Monitoring of Clinical Trials". Monitors under contract to the NIMH OCD ORO will visit the NIH site to monitor aspects of the study in accordance with the appropriate regulations and the approved protocol. The objectives of a monitoring visit will be: 1) to verify the existence of signed informed consent documents and documentation of the ICF process for each monitored subject; 2) to verify the prompt and accurate recording of all monitored data points, and prompt reporting of all SAEs; 3) to compare abstracted information from clinical databases (e.g. CTDB) with individual subjects' records and source documents (subjects' charts, laboratory analyses and test results, physicians' progress notes, nurses' notes, and any other relevant original subject information); and 4) to help ensure investigators are in compliance with the protocol. The monitors also will inspect the clinical site regulatory files to ensure that regulatory requirements (Office for Human Research Protections-OHRP), FDA, and applicable guidelines (ICH-GCP) are being followed. During the monitoring visits, the investigator (and/or designee) and other study personnel will be available to discuss the study progress and monitoring visit.

The investigator (and/or designee) will make study documents (e.g., consent forms, clinical database records and pertinent hospital/sources or clinical records readily available for inspection by the local IRB, FDA, the site monitors, and the NIMH staff for confirmation of the study data.

A specific protocol monitoring plan will be discussed with the Principal Investigator and study staff. The plan will outline the frequency of monitoring visits based on such factors as study enrollment, data collection status and regulatory obligations.

10.7 Quality Assurance and Quality Control

Each clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted, and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonization Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

10.8 Data Handling and Record Keeping

10.8.1 Data Collection and Management Responsibilities

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the PI. The PI is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents will be completed in a neat, legible manner to ensure accurate interpretation of data. Hardcopies of the study visit worksheets will be provided for use as source document worksheets for recording data for each participant enrolled in the study. Data recorded in the electronic case report form (eCRF) derived from source documents will be consistent with the data recorded on the source documents.

Clinical data (including AEs, eligibility, and primary outcome data) will be entered into the Clinical Trials Database (CTDB) at NIH. CTDB complies with the Federal Information Security Management Act of 2002 and 21 CFR Part 11. The data system includes audit trail, password protection, and control staff access level to the application and data. Edit checks implemented at the eCRF include: data type validation and numeric range checks. Clinical data will be entered directly from the source documents.

10.8.2 Study Records Retention

Study documents should be retained for a minimum of 2 years after the last approval of a marketing application in an International Conference on Harmonisation (ICH) region and until there are no pending or contemplated marketing applications in an ICH region or until at least 2 years have elapsed since the formal discontinuation of clinical development of the study intervention, and as per the NIH Intramural Records Retention Schedule. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

10.9 Protocol Deviations and Non-Compliance

The PI will use continuous vigilance to identify and report deviations and/or non-compliance to the NIH Institutional Review Board as per Policy 801. All deviations will be addressed in study source documents, reported to the NIMH Program Official and the IND sponsor; Dr. Maryland Pao, Clinical Director, holds both of these positions. The investigator will be responsible for knowing and adhering to the reviewing IRB requirements.

10.9.1 NIH Definition of Protocol Deviation

A protocol deviation is any changed, divergence, or departure from the IRB-approved research protocol.

- Major deviations: Deviations from the IRB approved protocol that have, or may have the potential to, negatively impact the rights, welfare or safety of the subject, or to substantially negatively impact the scientific integrity or validity of the study.
- Minor deviations: Deviations that do not have the potential to negatively impact the rights, safety or welfare of subjects or others, or the scientific integrity or validity of the study.

10.10 Publication and Data Sharing Policy

10.10.1 Human Data Sharing Plan

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

National Institutes of Health (NIH) Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals.

10.10.2 Genomic Data Sharing Compliance

No genomic data will be acquired during this study.

10.11 Conflict of Interest Policy

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the NIMH has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

11 ABBREVIATIONS

AE	Adverse Event
ANCOVA	Analysis of Covariance
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
CMP	Clinical Monitoring Plan
COC	Certificate of Confidentiality
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DSMB	Data Safety Monitoring Board
DRE	Disease-Related Event
EC	Ethics Committee
eCRF	Electronic Case Report Forms
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FFR	Federal Financial Report
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
GWAS	Genome-Wide Association Studies
HIPAA	Health Insurance Portability and Accountability Act
HSPU	Human Subjects Protection Unit
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDE	Investigational Device Exemption
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
ISO	International Organization for Standardization
ITT	Intention-To-Treat
LSMEANS	Least-squares Means
MedDRA	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures
MSDS	Material Safety Data Sheet
NCT	National Clinical Trial
NIH	National Institutes of Health
NIH IC	NIH Institute or Center
OHRP	Office for Human Research Protections
PI	Principal Investigator
QA	Quality Assurance

QC	Quality Control
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SMC	Safety Monitoring Committee
SOA	Schedule of Activities
SOC	System Organ Class
SOP	Standard Operating Procedure
UP	Unanticipated Problem
US	United States

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