Intranasal Insulin for Treating Posttraumatic Stress Disorder (PTSD)

1. Statement of Purpose:

Insulin is a hypoglycemic medication approved by the Food and Drug Administration (FDA) for the treatment of diabetes mellitus (DM). Recently several studies demonstrated that intranasal insulin can increase cognition and decrease stress-related behaviors, anxiety, and other psychiatric symptoms.[1, 2] Insulin modulates the neuronal synaptic plasticity through binding on the widely spread insulin receptors in the brain.[3-5] Recently, intranasal insulin has been suggested for the potential treatment of posttraumatic stress disorder (PTSD).[6] However, no studies have investigated the therapeutic role of intranasal insulin on PTSD yet. The current proposal aims to study the pharmacotherapeutic effects of intranasal insulin on PTSD symptoms (Aim-1), inhibition of amygdala hyperactivation (Aim-2), and reduction of anxiety (Aim-3) in subjects with PTSD. We hypothesize that intranasal insulin will reduce PTSD and anxiety symptoms by playing an integral role in glucose metabolism and restoration of insulin function in emotional and memory centers of the brain. In addition, data generated by functional magnetic resonance imaging (fMRI) studies of the amygdala during an emotional task will shed light on underlying mechanisms of action and the pharmacotherapeutic role of intranasal insulin.

Aim 1: To determine the pharmacotherapeutic role of intranasal insulin on PTSD symptoms.
Design: Twenty subjects with PTSD will be recruited to test intranasal insulin as a potential agent for PTSD treatment in a double-blind, crossover, placebo-controlled pilot study. PTSD symptoms will be determined by the Clinician Administered PTSD Scale (CAPS).
Hypothesis: Intranasal insulin is superior to placebo in reducing PTSD symptoms.

Aim 2: To demonstrate the inhibiting effects of intranasal insulin on hyperactivation of amygdala.
***Design*:** Regular fMRI images, with the amygdala used as the region of interest (ROI), will be taken after administration of intranasal insulin/placebo during an emotional task during the study.
Hypothesis: Intranasal insulin is superior to placebo in reducing amygdala hyperactivation during an emotional task.

Aim 3: To study the effect of intranasal insulin on anxiety symptoms in PTSD subjects.
Design: Anxiety-related symptoms will be recorded by the Hamilton Anxiety Rating Scale (HAM-A).
***Hypothesis*:** Intranasal insulin is superior to placebo in reducing anxiety symptoms.

2. Background:

Significance

PTSD has surfaced as a serious public health problem with lifetime prevalence rates of 7.8% in the general US population, 27-31% among Vietnam veterans, 12% in Iraq and Afghanistan veterans,[7] and 12.8% in Gulf war veterans.[8] Combat PTSD has strong association with poor quality of health.[7] Veterans with PTSD have higher rates of depression (Iraq veterans 6.6%, Afghanistan veterans 6.9%),[7] more missed workdays (Afghanistan veterans),[9] higher risk of alcohol abuse (33% Iraq and Afghanistan veterans), [7] higher somatic problems (Gulf

veterans),[8, 10] and poorer physical quality of life (WWII and Korean Veterans)[11] compared to Veterans without PTSD. PTSD is the leading factor of higher mental healthcare utilization in US Veterans[7] and imposes risk of suicide attempts 6 times higher in general US population.[12] Despite enormous morbidity and mortality associated with PTSD, the efficacy of current pharmacotherapy is only modest, especially among Veterans.[13, 14] Despite the disabling and chronic nature of PTSD, only two medications (paroxetine and sertraline), both selective serotonin reuptake inhibitors (SSRIs), have been approved by the FDA for PTSD.[15-18] However, accumulating evidence highlights the limitation of SSRI treatment and the increasing prevalence of SSRI-resistant PTSD.[19, 20] Thus, the development of effective pharmacotherapy for PTSD has been an initiative, strongly supported by the Department of Veterans Affairs, the Department of Defense, the National Institute of Mental Health, and the Institute of Medicine.

Innovation

This is the first study investigating the effects of intranasal insulin in subjects with PTSD. Intranasal administration of medications bypasses the blood brain barrier (BBB) by travelling extracellularly along the olfactory and trigeminal neurons. This route has been studied in Alzheimer's disease,[3] mood disorder,[21] Huntington's disease,[2] cerebrovascular accidents,[22] and focal cerebral ischemic attacks.[22, 23] In the last decade, intranasal insulin has emerged as a potential pharmacotherapeutic agent in the treatment of CNS diseases.[5] Insulin receptors are distributed on both neurons and astrocytes with the highest density on the olfactory bulb, cerebral cortex, hippocampus, hypothalamus, amygdala, and cerebellum.[4, 5, 24-28] Intranasal insulin increases dendritic plasticity and synaptic density as well as glucose utilization of neuronal network.[3] It may play an active role by correcting metabolic imbalances and restoring normal insulin functions in brain. Phase II clinical trials have demonstrated that intranasal insulin improved memory in both Alzheimer's disease patients [29, 30] and healthy adults.[31] The recent popularity of intranasal insulin is due to the following reasons: 1) practical, convenient, and cost-effective treatment, 2) non-invasive administration, 3) excellent safety profiles, 4) direct delivery to the central nervous system (CNS), 5) rapid delivery to the CNS within minutes, 6) low unwanted systemic side effects by targeting the brain without affecting peripheral blood levels of insulin or glucose, and 7) no need to modify insulin for intranasal delivery.[32] To date, no studies have tested intranasal insulin in PTSD population. In this study, we will investigate the pharmacotherapeutic role of intranasal insulin in PTSD subjects.

Background

Mounting evidence indicates that intranasal insulin may reduce anxiety or stress-related behaviors. Down-regulation of hypothalamic insulin receptors increases anhedonia and anxiety-like behaviors, suggesting effects of insulin on emotion.[33]

Animal Studies

The hyperphosphorylation of tau protein causes the accumulation of neurofibrillary tangles in neurons and leads to the memory and cognition impairment.[34-36] Large number of studies tested intranasal insulin to understand the effects of insulin on molecular levels.[25, 28, 37-41] In a study using knockout rats, intranasal insulin reduced hyperphosphorylation of tau proteins, while subcutaneous insulin had minimum effects on hyperphosphorylation of tau protein.[38, 39] Intranasal insulin also increased levels of synaptic proteins, reduced A-Beta40 protein (leading cause of Alzheimer's disease) level, and restoration of insulin signaling.[38] Intranasal insulin increased short- and long-term object recognition and reduced anxiety behavior in

mice.[28] These studies provide evidence that intranasal insulin improves memory and cognition and reduces anxiety.

Human Studies
Numerous studies had investigated intranasal technique in humans with intranasal insulin spray. Different doses of intranasal insulin (20 IU, 40 IU, or 60 IU) have been administered for 2 weeks, 8 weeks, or 16 weeks in healthy volunteers and in psychiatric patients without adverse effects.[1, 42] Intranasal insulin elevates the memory and cognition without any impact on blood insulin and sugar levels.[42, 43] In healthy subjects, a single dose of 40 IU of intranasal insulin enhanced self-confidence, raised feelings of wellbeing, and reduced anger,[31] while 20-40 IU of intranasal insulin for 8 weeks or 16 weeks increased cognitive abilities, self-confidence, and memory in subjects with Alzheimer's disease or mild cognitive impairment (MCI).[1, 3, 31, 41, 44] Intranasal insulin increases energy metabolism, regional blood flow, neuronal plasticity, and neuronal survival.[3] A single dose of 40 IU intranasal insulin significantly decreases cortisol response to Trier Social Stress Test (TSST).[31] This reflects insulin can stabilize hypothalamic-pituitary-adrenal (HPA) axis response to psychosocial stress.

Although intranasal insulin had not been tested in PTSD subjects, the findings from both animal and human studies suggest that it may be effective in reducing PTSD symptoms. The main neurobiological features of PTSD include amygdala hyperactivity and alteration of the HPA axis. Given the hyper-responsiveness of the HPA axis in PTSD, intranasal insulin may decrease hyper-arousal symptoms of PTSD by stabilizing the HPA axis. In addition anxiolytic properties of intranasal insulin[28] may be beneficial for PTSD subjects.[6]

In this pilot study, we will test intranasal insulin as a potential agent for PTSD treatment. We will also evaluate whether intranasal insulin inhibits the hyperactivation of amygdala during an emotional faces viewing task, as this may be a more sensitive biomarker of treatment effects than symptom rating scales.

3. Research Plan:

A. Overview

In this double-blind, crossover, placebo-controlled pilot study, 20 subjects with a current PTSD will receive 40 IU of intranasal insulin or placebo twice a day (80 IU per day). fMRI will record inhibiting effects of drug on amygdala hyperactivation at three points (visit-2, visit-3 and visit-5) during study period. Insulin may help with the resolution of PTSD symptoms by increasing the dendritic plasticity at brain emotion center, the amygdala. Data obtained from this pilot project will be statistically analyzed to determine the pharmacotherapeutic role of intranasal insulin on amygdala hyperactivation, PTSD, and anxiety-related symptoms.

B. Experimental Subjects

Twenty subjects with PTSD between the ages of 21-65 years will be recruited for this study through advertisement and referral from clinics. Subjects will complete an informed consent process and will be thoroughly screened for inclusion and exclusion criteria as described below.

B.1. Recruitment and Informed Consent

Subjects will be recruited in traditional ways through flyers, posters, and research boards at the VA Connecticut Healthcare System. There will be no web applications. Subjects will be identified via their response to advertisements and/or internal recruiting through the research clinics, mental health clinics and medical record review. If a subject is identified through medical record review their physician/clinician will be contacted by research staff to explain the study and ask best way to connect with subject. Subjects will be asked to call us (West Haven VA Alcohol Research Center) at the number provided on the flyers if they are interested in participating in the research study. Subjects will be briefly screened over the phone for eligibility, and, if the subject seems suitable, he or she will present for an in-person evaluation that will be preceded by the consent process. All information obtained for the purposes of recruiting subjects, completing questionnaires, and processing data are confidential. Designated research staff will be responsible for recruiting potential subjects.

B.2. Discontinuation of subjects

Criteria for discontinuation
Subjects may be discontinued from study treatment and assessments at any time. Specific reasons for discontinuing a subject from this study are:

- Voluntary discontinuation by the subject, who is at any time free to discontinue his/her participation in the study without prejudice to further treatment.
- Clinical deterioration as judged by the investigator.
- Safety reasons as judged by the investigator.
- Severe non-compliance to protocol as judged by the investigator.
- Incorrect enrollment i.e., the subject does not meet the required inclusion criteria for the study.

Subject lost to follow-up
A subject who discontinues will always be asked about the reason(s) for discontinuation and the presence of any adverse events. If possible, they will be seen and assessed by the investigator. Adverse events will be followed up as medically necessary.

C. Pre-study screening procedure and initial assessments

After an initial phone screen to determine obvious exclusions from the study protocol, potential subjects will be invited for a clinic prescreen and personal interview. All subjects will have an initial screening assessment that includes medical history, psychiatric history, and standardized psychiatric/medical assessments.

D. Screening

D.1. Screening physical and laboratory workup

All subjects will have a standard physical examination at the time of the initial screening. Routine laboratory studies include CBC with differential, electrolytes, and liver function tests. A urine sample will be collected for urine toxicology, pregnancy, and urinalysis. All female subjects of child-bearing potential will complete a urine pregnancy test prior to participating in the studies.

D.2. Screening psychiatric assessments

All subjects will have a psychiatric interview conducted by a study clinician. In addition to the standard psychiatric examination, subjects will receive a structured diagnostic interview – the Structured Clinical interview for DSM-5 (SCID) and CAPS. Baseline and follow-up ratings will also be obtained via assessment measures (see Table 1).

Table 1

Baseline Assessments	**Medical Assessments**	**Rating Scales**
Demographic Data	Vital signs	CAPS
Psychiatric History	Physical Examination	PCL
Medial History	Laboratory Assessment	SCID
		HAM-A
		CGI/PGI
		MARDS

Abbreviations: Clinical Global Impression (CGI), Patient Global Impression (PGI), Hamilton Anxiety Rating Scale (HAM-A), Montgomery-Asberg Depression Rating Scale (MADRS), Structured Clinical Interview for DSM Disorders (SCID), functional Magnetic Resonance Imaging (fMRI).

D.3. Historic and demographic information

Each subject will complete questionnaires about the subjects' demographics including education level, socioeconomic status, occupation, race, ethnicity, and family history of mental illness.

E. Ongoing clinical assessments

E.1. Medical assessment: Physical examination (including vital sign determination) and clinical laboratories will be completed at the first visit.
E.2. Psychiatric assessment: Ratings are performed by trained research staff. The primary investigator supervises the administration of clinical measures. Each of the psychiatric assessment instruments is briefly described below.

F. Psychological Evaluation:

1. *Clinician Administered PTSD Scale (CAPS):* The CAPS is a standardized clinician-rated instrument to assess the presence and severity of PTSD symptoms.
2. *Clinical Global Impressions Scale (CGI)* and the *Patient Global Impressions Scale (PGI):* The CGI and PGI are widely used instruments, which assess overall severity of illness on a 1 to 7-point scale with 1 indicating "normal, not at all ill" and 7 indicating "among the most extremely ill patients." These instruments also assess global improvement on a 1-to-7-point scale with 1 indicating "very much improved," 4 indicating "no change" and 7 indicating "very much worse."
3. *Hamilton Anxiety Rating Scale (HAM-A):* The HAM-A is a standardized clinician-rated instrument to evaluate the severity of anxiety symptoms.
4. *Montgomery-Asberg Depression Rating Scale (MADRS):* The MADRS is a standardized instrument to ascertain depressed mood and neurovegetative signs and symptoms of depression.
5. *PTSD Checklist (PCL):* The PCL is used to measure PTSD symptoms and is a self-report questionnaire that shares similar reliability with the CAPS.

6. *Structured Clinical Interview for DSM-5 (SCID)* will be used to provide current and past diagnoses of psychiatric disorders, notably the inclusion/exclusion criteria of a current major depressive episode.
7. *Socio-demographic/General Information:* At intake, demographic data and medical history will be assessed with interviews and self-report forms that provide data on age, race, socioeconomic status, marital status, educational and occupational levels.

Table 2 Study events at each visit

Visit	Visit 1 (Screening)	Visit 2	Visit 3	Visit 4	Visit 5
Day	-30-0	0+	7+	14+	21+
fMRI		X	X		X
Intranasal Insulin/placebo		X		X	
Informed consent*	X				
Inclusion/Exclusion	X				
SCID	X				
Socio-Demographics	X				
Medical history	X				
Psychiatric history	X				
Physical	X				
Vitals	X	X	X	X	X
Adverse Event Log	X	X	X	X	X
Urinalysis/CBC/ Chemistry 7/ Blood insulin	X				
Urine toxicology/pregnancy	X				
MADRS	X	X	X	X	X
HAM-A	X	X	X	X	X
CGI/PGI		X	X	X	X
PCL	X	X	X	X	X
CAPS	X	X	X	X	X
Blood glucose (finger stick)		X	X	X	X

*Informed consent will be obtained prior to any study procedure.

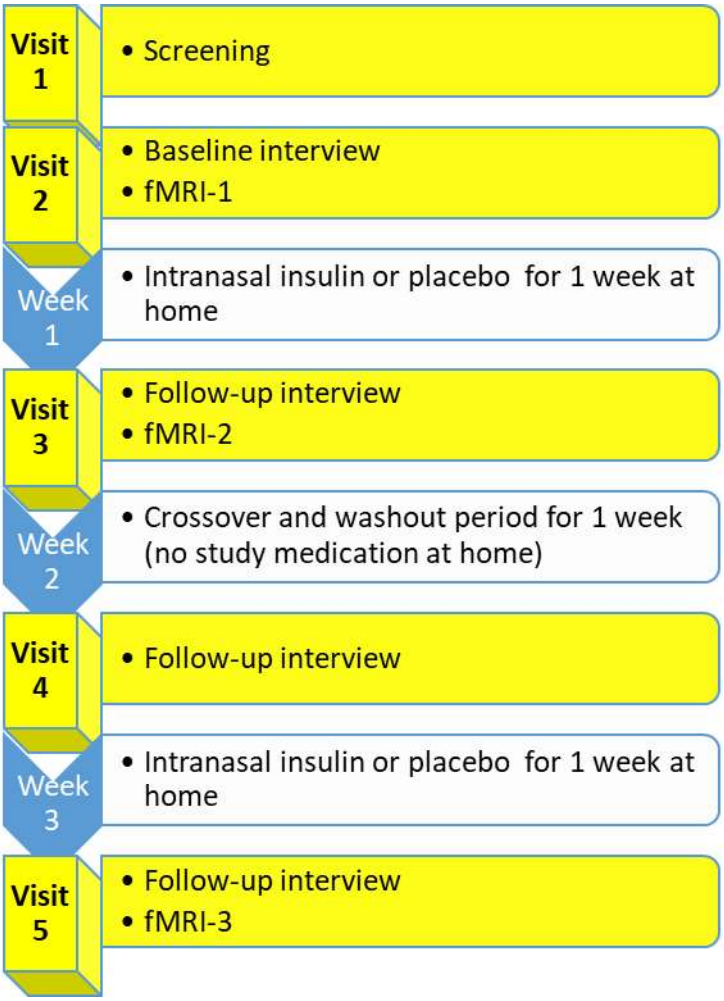
G. Insulin

Subjects will be randomly assigned to one of two treatment conditions (A or B). Subjects will take either 40 IU of insulin twice a day (80 IU per day) or placebo for one week for each drug, separated by a washout period (study drug free period) for 1 week (Table 3).

Table 3

Condition	Study Medications		
	Week 1	Week 2	Week 3
A	Intranasal insulin x 1 week	No medication x 1 week	Placebo x 1 week
B	Placebo x 1 week	No medication x 1 week	Intranasal insulin x 1 week

H.1. Study Design



H.2. Visit 1 (Day -30-0)

Screening

At the first visit, research staff will meet the potential subjects at VA Connecticut Healthcare System, 950 Campbell Ave West Haven, CT 06516. After the screening, staff will discuss the nature and the benefits/risks of the study with the eligible subjects before the informed consent process. This visit will take approximately 4-5 hours to complete all procedures (medical and psychiatric evaluation, labs, clinical assessments, etc.). Eligible and consented subjects will be scheduled for their first fMRI study visit.

H.2. Visit 2 (Day 0+)

fMRI-1

At the second visit, subjects can choose to meet the research staff VA Connecticut Healthcare System in West Haven, 950 Campbell Ave and be escorted to Yale MRRC, 300 Cedar street in New Haven or if subject familiar with the area, she/he may choose to meet staff at Yale MRRC facility directly. After randomization, research staff will administer clinical assessment measures to monitor symptoms on the day of the scan. All subjects will undergo fMRI-1, an emotional fMRI paradigm (see below). After fMRI-1, all subjects will receive study medication (either insulin or placebo) so that he/she can take study medication at home until the next visit. Intranasal insulin dose will be 40 IU twice

a day. The first dose (either insulin or placebo) will be administered by the subject in front of research physician. The subject will be monitored for 60-90 minutes for any adverse events. One baseline blood glucose (before intranasal insulin) and one post-dose blood glucose (between 60-90 minutes) will be measured with fingerstick blood glucose testing. We will educate all subjects about how to recognize the signs and symptoms of hypoglycemia (after using intranasal insulin) and how to correct hypoglycemia (by eating or drinking a small amount of glucose-rich food, such as sugar, fruit juice, or hard candy). This visit will take approximately 3-4 hours to complete.

H.3. Visit 3 (Day 7+)

fMRI-2

At the third visit, subject will report at Yale MRRC facility, 300 Cedar Street New Haven, CT 06519. Research staff will collect the study drug and administer clinical measures. All subjects will undergo fMRI-2. After the fMRI-2, there will be a 1-week crossover and washout period during which subjects will not take study drug. This visit will take 3-4 hours approximately.

H.4. Visit 4 (day 14+)

Fourth visit does not include fMRI session and will be conducted at VA Connecticut Healthcare System in West Haven. Research staff will administer clinical measures. As this is a crossover study, those subjects who receive insulin on Day 0 will receive placebo on Day 14. Those who receive placebo on Day 0 will receive insulin on Day 14 to use at home. All subjects will receive study medication (either insulin or placebo) so that he/she can take study medication at home until the next visit. Intranasal insulin dose will be 40 IU twice a day. The first dose (either insulin or placebo) will be administered by the subject in front of research physician. The subject will be monitored for 60-90 minutes for any adverse events. One baseline blood glucose (before intranasal insulin) and one post-dose blood glucose (between 60-90 minutes) will be measured with fingerstick blood glucose testing. This visit will take 90 minutes approximately.

H.5. Visit 5 (day 21+)

fMRI-3

At the fifth and the last visit, subjects will report at Yale MRRC in New Haven. Research staff will collect study drug and administer clinical measures. All subjects will undergo fMRI-3. This visit will take 3-4 hours approximately.

I. fMRI Paradigm

Scans will be collected on a Siemens TIM Trio A 3.0 Tesla MRI scanner located at the Yale Magnetic Resonance Research Center (MRRC), using standard MRRC sequences. The scan protocol consists of a 3-plane localizer collected from three cardinal planes. These images serve as a localizer series for the other acquisitions. The remaining multi- modal magnetic resonance pulse sequences may include: two high resolution T1-weighted imaging (MPRAGE or FLASH); high resolution T2-weighted imaging (SPACE); three- dimensional chemical shift imaging (CSI) provides high signal to noise 1H spectra; BOLD gradient echo planar imaging at rest and/or during FMRI tasks (e.g. trauma-related cues); high angular diffusion weighted imaging, arterial spin labeling (ASL), susceptibility weighted imaging, and ultra-high resolution T1 anatomical imaging (400 micron x 400 micron x 800 micron). FMRI tasks will be back-projected onto a screen by a high contrast projector connected to a laptop computer. Behavioral responses will be recorded during FMRI tasks with an optical response system. During the MRI scan, participants will be asked to perform certain tasks. They will be given detailed instructions by the experimenter explaining the task instructions. During some of the cognitive tasks they may see

various distracting images some of which will be photographs. As in the behavioral assessment portion, the photographs that they will see may cover a broad range of contents, including slides of flowers, children, families, people's faces, insects, animals, sports, and graphic slides similar to those that might be seen in a newscast or documentary of war footage (including mutilated individuals and dead bodies). Many people experience some degree of distress when viewing the most graphic pictures. If they will be shown these graphic images they will have an opportunity to view some sample pictures to make certain that they understand the types of slides they will see during the study and the level of intensity that the pictures may contain. They will be able to choose if they are comfortable with each level of intensity. If they agree to look at the pictures and become upset during the scanning they may indicate their distress and the pictures will be stopped. We will remind them that they may ask to stop viewing the pictures at any time during the testing and still continue in the study.

We will use HEA and HEP coil element and will acquire the following scans:01 - 3-Plane Localizer (FOV=280mm)
02 - T1-Weighted Saggital 3D MPRAGE (FOV=256mm)
03 - T2-Weighted SPACE (FOV=256mm)
04 - Field Map (Multi-band 6; FOV=210mm)
05 - Field Map (Multi-band 6; FOV=210mm)
06 - Multi-band Echo Pulse (2d; Anterior-to-Posterior; FOV=210mm)
07 - Multi-band Echo Pulse (2d; Posterior-to-Anterior; FOV=210mm)
08 - BOLD rs-fMRI Single Band Reference (FOV=210mm)
09 - BOLD rs-fMRI (2.5mm; 0.7TR; Multi-band 6; FOV=210mm)
10 - BOLD task-fMRI Single-band Reference (FOV=210mm)
11 - BOLD task-fMRI (2.5mm; 0.7TR; Multi-band 6; FOV=210mm)
12 - BOLD rs-fMRI Single Band Reference (FOV=210mm)
13 - BOLD rs-fMRI (2.5mm; 0.7TR; Multi-band 6; FOV=210mm)
14 - DWI 90 Single-band Reference (Right-to-Left; 1.8mm; FOV=216mm)
15 - DWI 90 (Multi-band; 1.8mm; Right-to-left; FOV=216mm)
16 - DWI 90 ADC Map (Multi-band; 1.8mm; Right-to-left; FOV=216mm)
17 - DWI 90 Trace-weighted Map (Multi-band; 1.8mm; Right-to-left; FOV=216mm)
18 - DWI 90 FA Map (Multi-band; 1.8mm; Right-to-left; FOV=216mm)
19 - DWI 90 ColFA Map (Multi-band; 1.8mm; Right-to-left; FOV=216mm)
20 - DWI 90 Single-band Reference (Left-to-Right; 1.8mm; FOV=216mm)
21 - DWI 90 (Multi-band; 1.8mm; Left-to-Right; FOV=216mm)
22 - DWI 90 ADC Map (Multi-band; 1.8mm; Left-to-Right; FOV=216mm)
23 - DWI 90 Trace-weighted Map (Multi-band; 1.8mm; Left-to-Right; FOV=216mm)
24 - DWI 90 FA Map (Multi-band; 1.8mm; Left-to-Right; FOV=216mm)
25 - DWI 90 ColFA Map (Multi-band; 1.8mm; Left-to-Right; FOV=216mm)
26 - DWI 91 Single-band Reference (Right-to-Left; 1.8mm; FOV=216mm)
27 - DWI 91 (Multi-band; 1.8mm; Right-to-left; FOV=216mm)
28 - DWI 91 ADC Map (Multi-band; 1.8mm; Right-to-left; FOV=216mm)
29 - DWI 91 Trace-weighted Map (Multi-band; 1.8mm; Right-to-left; FOV=216mm)
30 - DWI 91 FA Map (Multi-band; 1.8mm; Right-to-left; FOV=216mm)
31 - DWI 91 ColFA Map (Multi-band; 1.8mm; Right-to-left; FOV=216mm)
32 - DWI 91 Single-band Reference (Left-to-Right; 1.8mm; FOV=216mm)
33 - DWI 91 (Multi-band; 1.8mm; Left-to-Right; FOV=216mm)
34 - DWI 91 ADC Map (Multi-band; 1.8mm; Left-to-Right; FOV=216mm)
35 - DWI 91 Trace-weighted Map (Multi-band; 1.8mm; Left-to-Right; FOV=216mm)
36 - DWI 91 FA Map (Multi-band; 1.8mm; Left-to-Right; FOV=216mm)
37 - DWI 91 ColFA Map (Multi-band; 1.8mm; Left-to-Right; FOV=216mm)

38 - T1-Weighted FLASH (20 slices; FOV=256mm)
39 - PASL Post-label delay (20 slices; FOV=256mm)
40 - PASL Echo planar image (2d; 20 slices; FOV=256mm)
41 - PASL Echo planar image (2d; 20 slices; FOV=256mm)

Participants may be asked to return for a repeat session due to inadequate image acquisition or time constraints. No more than three repeat sessions will be attempted.

Emotion-regulation task:
Emotion-regulation tasks allow for assessment of neural networks engaged during automatic (implicit) and voluntary (explicit) emotional processing.

Emotion-regulation task: During task performance participants are presented with a series of stimuli taken from the International Affective Picture System (IAPS) that have been previously validated as either negative or neutral. Prior to each stimulus, participants are presented with a fixation cross, followed by an instruction to either "LOOK" (attend to stimulus) or "DECREASE" (decrease emotion via reappraisal of stimulus). Following each stimulus, participants rate their emotional reaction using a Likert-type scale via a button box. Prior to scanning, all participants are given training in negative emotion-regulation strategies using previously validated methods (Ochsner, K. N. et al. For better or for worse: neural systems supporting the cognitive down- and up-regulation of negative emotion. Neuroimage 23, 483-499, doi:10.1016/j.neuroimage.2004.06.030 (2004).)

4. Subject Population:

Twenty subjects, between the ages of 21-65 with current PTSD will be recruited. Subjects will be accepted into the protocol after an opportunity to review and provide voluntary written informed consent (see attached) and completion of a medical/psychiatric history, physical examination, and routine laboratory assessments. Participants will be enrolled if they meet the study criteria. Additional demographic, diagnostic and other clinical data will be obtained during the face-to-face clinical assessment. All such data will be recorded in written and/or electronic format(s).

Inclusion Criteria

- Male or female, 21-65 years old
- Current PTSD
- Able to provide written informed consent

Exclusion Criteria

- Unstable medical condition requiring acute new intervention
- Diabetes requiring insulin or oral hypoglycemic agents
- Moderate-severe traumatic brain injury
- Current psychotic disorder, bipolar disorder, cognitive disorder in the past 12 months
- Current substance use disorders (except alcohol, tobacco, or cannabis) in the past 3 months
- Changes in doses of psychotropic medications in the past 4 weeks
- Initiation of individual therapy or counseling in the past 4 weeks
- Imminent suicidal or homicidal risk
- Contraindication to Insulin
- History of claustrophobia
- Presence of cardiac pacemaker or other electronic device or ferromagnetic metal foreign

bodies in vulnerable positions as assessed by a standard pre-MRI safety screening questionnaire

- ❖ Pregnant or nursing women, positive pregnancy test, or inadequate birth control methods in women of childbearing potential

Subject eligibility will first be assessed via telephone screening. If the subject seems to be a likely candidate for inclusion in this protocol, he/she will be invited to study site and evaluated for study eligibility in person. Telephone screens will occur by experienced research personnel adept with this process. Suitability for enrollment will be assessed by experienced research personnel via face-to-face evaluation.

The risks involved with this study are the risks associated with intranasal insulin, MR study, phlebotomy/ blood draw, clinical assessments, and psychiatric clinical deterioration.

A. Intranasal Insulin

Insulin is a hypoglycemic medication to control blood sugar in diabetes mellitus (DM). Many generations of insulin have been produced since its discovery in 1920. In this study we will use Novolin (regular insulin), which is approved by the FDA for treating DM. Also, Novolin is the type of insulin that has been used safely and most often in the 11 published human clinical trials using intranasal insulin to improve memory in both patients with AD or MCI and in normal healthy adults.

Side effects of insulin depend on the dose, type, and route of administration. The most common side effects after subcutaneous administration include: hypoglycemia, hypokalemia (low potassium level in blood), local allergic reaction, and weight gain. Moderate hypoglycemia may cause perspiration, drowsiness, and slow reflexes. In general, moderate hypoglycemia is transient and self-tolerant. Severe hypoglycemia may cause loss of consciousness and convulsions. Insulin should be used cautiously in individuals who are predisposed to hypoglycemia, like individuals who are fasting or have erratic food intake. Severe hypokalemia may cause respiratory muscles weakness or ventricular arrhythmia. Local redness, irritation, and itching are the signs of local allergy. These allergic reactions usually subside with time and rarely need discontinuation of insulin. Long term, for years, use of insulin may cause weight gain. Insulin is pregnancy category B drug, mean it is safe to use during pregnancy.[45] The studies completed in humans did not report teratogenic effects while animal studies did. Insulin is gold standard medication for management of gestational diabetes and DM not responding to nutritional modifications. Nonetheless, insulin causes above mentioned side effects, except local irritation, once significant dose of insulin enters into systemic blood circulation. The intranasal route proposed in this study bypasses systemic circulation and it has no effect on systematic blood sugar and insulin levels.[42, 43]

B. MR Studies

Magnetic resonance (MR) is a technique that uses magnetism and radio waves, not x- rays, to take pictures and measure chemicals of various parts of the body. The United States Food and Drug Administration (FDA) has set guidelines for magnet strength and exposure to radio waves, and we carefully observe those guidelines.

Subjects will be watched closely throughout the MR study. Some people may feel uncomfortable or anxious. If this happens to a subject, they may ask to stop the study at any

time and we will take them out of the MR scanner. On rare occasions, some people might feel dizzy, get an upset stomach, have a metallic taste or feel tingling sensations or muscle twitches. These sensations usually go away quickly but subjects are instructed to tell the research staff if they have them.

MR Task: During the MR scan, participants will be asked to perform certain tasks. They will be given detailed instructions by the experimenter explaining the task instructions. During some of the cognitive tasks they may see various distracting images some of which will be photographs. As in the behavioral assessment portion, the photographs that they will see may cover a broad range of contents, including slides of flowers, children, families, people's faces, insects, animals, sports, and graphic slides similar to those that might be seen in a newscast or documentary of war footage (including mutilated individuals and dead bodies). Many people experience some degree of distress when viewing the most graphic pictures. If they will be shown these graphic images they will have an opportunity to view some sample pictures to make certain that they understand the types of slides they will see during the study and the level of intensity that the pictures may contain. They will be able to choose if they are comfortable with each level of intensity. If they agree to look at the pictures and become upset during the scanning they may indicate their distress and the pictures will be stopped. We will remind them that they may ask to stop viewing the pictures at any time during the testing and still continue in the study.

There are some risks with an MR study for certain people. Applicants may be excluded if they have a pacemaker or some metal objects inside their body because the strong magnets in the MR scanner might harm them. Another risk is a metallic object flying through the air toward the magnet and hitting the subject. To reduce this risk we require that all people involved with the study remove all metal from their clothing and all metal objects from their pockets. Subjects will walk through a metal detector prior to entering the magnet room. Nothing metal can be brought into the magnet room at any time. Also, once the subject is in the magnet, the door to the room will be closed so that no one from outside accidentally goes near the magnet.

Subjects are instructed to read and answer very carefully the questions on the MR Safety Questionnaire related to their personal safety. They are instructed during the consent process to take a moment to be sure that they have read the MR Safety Questionnaire and to be sure to tell the staff any information they think might be important.

This MR study is for research purposes only and is not in any way a clinical examination. The scans performed in this study are not designed to find abnormalities. The primary investigator, the lab, and the Magnetic Resonance Research Center are not qualified to interpret the MR scans and are not responsible for providing a diagnostic evaluation of the images. If a worrisome finding is seen on a subject's scan, a radiologist or another physician will be asked to review the relevant images. Based on his or her recommendation (if any), the primary investigator or consulting physician will contact the subject, inform them of the finding, and recommend that they seek medical advice as a precautionary measure. The decision for additional examination or treatment would lie solely with the subject and their physician. The investigators, the consulting physician, the Magnetic Resonance Research Center, and Yale University are not responsible for any examination or treatment that the subject receives based on these findings. The images collected in this study are not a clinical MR exam and for that reason, they will not be made available for diagnostic purposes.

C. Clinical Assessments

These clinical Assessments are all non-invasive, add no risk, and have been used without difficulty or adverse events in our previous studies with a similar population. The participants may experience couple of disadvantages including: (1) these assessments take time to complete, and (2) questions about their past including potentially sad and traumatic events may upset them. Our past experience with these assessments indicates that these are acceptable to participants and they rarely feel unpleasant at times. Only subjects' code numbers will be recorded on the forms themselves to protect confidentiality.

D. Psychiatric Clinical Deterioration

There is a risk of increasing PTSD symptoms due to the natural history of the illness.

E. Phlebotomy/blood draw

Blood drawing may cause pain, bruising, and rarely fainting or infection. These risks are minimized by proper techniques. Approximately two teaspoonfuls of blood will be withdrawn at screening visit.

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

A. Recruitment and Informed Consent

Subjects will be recruited from the Yale-New Haven area by advertisements and by referrals from clinicians. Prior to enrollment, subjects will have face-to-face interviews with research staff where the nature of the project, the risks, the benefits, and the alternatives to participation will be discussed with the subject and, if necessary, the subject's family. If the subject continues to be interested in the project, informed written consent will be obtained on the consent form approved by the Yale University Human Investigation Committee (HIC).

B. Protection Against Risk

General risk-minimizing strategies: **1.** Effective screening to exclude subjects who would be placed at a greater risk. This includes a comprehensive psychiatric evaluation, medical evaluation, and physical examination. Trained staff, under the supervision of the PI, will conduct all screening procedures. **2.** The investigator or research staff will explain the benefits and risks of participation in the study to each subject. Subjects will be asked to verbalize their understanding of all aspects of the consent, including risks, benefit and alternatives. The voluntary nature of research studies is always emphasized. **3.** All subject information will be kept confidential and only members of the investigative team with appropriate IRB/HIC and HIPAA training will have access to the study data. Data will be maintained and secured in locked file cabinets or password protected electronic media. A numbering code will be used to assign a unique identifier to each subject. **4.** A Data and Safety Monitoring Plan is described below.

C. Metal Objects

To minimize the chance of a magnetic object entering the magnet room the following precautions are taken:

1. All personnel involved in the study must go through training provided by the MRRC.
2. The subject is screened in the MRRC for metal objects prior to being allowed into the magnet room.
3. Once the subject is in the room only the research team members who have successfully completed the MRRC safety requirements will be allowed into the room.

If an accident occurs, 911/EMS will be activated. All personnel are trained in an established procedure for removing the subject from the magnet. Due to the close proximity to the hospital the time required for the ambulance team to arrive will be under 5 minutes. All personnel involved in the study are trained in this procedure. There will be a clinician present at all times who will be able to perform immediate resuscitation in the unlikely event it is needed.

D. Subject Monitoring and Emergency Procedures

An experienced clinical research team will closely monitor the subjects. All of the information obtained from participants in this study will be coded by numbers and kept in locked files in the research unit to ensure confidentiality. At least one member of the research team will watch the subject while in the magnet room. Visual and verbal contact will be maintained throughout the imaging. Subjects who become claustrophobic in the magnet will be removed immediately from the magnet room. Subjects who experience distress in the magnetic room for any reason including seizures, loss of consciousness, pain will be removed from the magnet room in accordance with well-established procedures developed for the MRRC. All team members will be thoroughly familiar with the established emergency plan. A nonmagnetic stretcher will be on standby next to the entrance to the magnet. Nonmagnetic oral airways, Ambubag, and stethoscope are stored with the emergency stretcher in the magnet room. Should an emergency arise, one member of the team will telephone the 911/EMS operator and have an ambulance with personnel trained in resuscitation sent to the MRRC. The other members will remove the patient via stretcher from the magnet room taking the patient to the part of the MRRC designated for cardiopulmonary resuscitation. If immediate resuscitation is needed the physician present during the study will perform this procedure.

E. Anxiousness

If a subject is anxious and expresses a desire to leave the magnet room, the study will be immediately terminated.

F. Hypoglycemia

Although the risk of hypoglycemia is low, a study clinician trained to recognize signs of hypoglycemia will be present during study and will give oral sugar (high-sugar foods) to correct the blood sugar level if needed. Blood sugar level (finger stick) will be monitored in the study. If subjects have (1) a blood sugar of 70 or less or (2) hypoglycemic symptoms (sweating, confusion, shakiness, irritability, severe anxiety, pale skin) after receiving intranasal insulin, subjects will receive oral sugar. Subjects will be withdrawn from the study if they become hypoglycemic or experience a 30 mg/dL drop in blood sugar after receiving intranasal insulin.

6. Data and Safety Monitoring Plan: Include an appropriate Data and Safety Monitoring Plan (DSMP) based on the investigator's risk assessment stated below. (Note: the HIC will

make the final determination of the risk to subjects.) For more information, see the Instructions, page 24.
a. What is the investigator's assessment of the overall risk level for subjects participating in this study? The investigator's assessment of the overall risk level for subjects participating in this study is moderate.
b. If children are involved, what is the investigator's assessment of the overall risk level for the children participating in this study? Not Applicable
c. Copy, paste, and then tailor an appropriate Data and Safety Monitoring Plan from http://www.yale.edu/hrpp/forms-templates/biomedical.html for
i. Minimal risk
ii. Greater than minimal/moderate risk
iii. High risk

d. For multi-site studies for which the Yale PI serves as the lead investigator: Not Applicable

Greater than minimal Risk DSMP

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

The principal investigator, Gihyun Yoon, M.D., will be responsible for monitoring the data, assuring protocol compliance, and conducting the safety reviews at the specified frequency which must be conducted at a minimum of every 6 months (including when reapproval of the protocol is sought). During the review process, the principal investigator (monitor) will evaluate whether the study should continue unchanged, require modification/amendment, continue or close to enrollment. Either the principal investigator or the HIC have the authority to stop or suspend the study or require modifications.

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

1. We do not view the risks associated with insulin as minimal.
2. We do not view the risks associated with the combined use of insulin and MR imaging as minimal.
3. Given the now established safety and validity of Insulin in prior work, we do not view the proposed study as high risk.

Although we have assessed the proposed study as one of moderate risk, the potential exists for anticipated and/or unanticipated adverse events, serious or otherwise, to occur since it is not possible to predict with certainty the absolute risk in any given individual or in advance of firsthand experience with the proposed study methods.

Therefore, we provide a plan for monitoring the data and safety of the proposed study as follows:

3. Attribution of Adverse Events:

Adverse events will be monitored for each subject participating in the study and attributed to the study procedures / design by the principal investigator, Gihyun Yoon, M.D., according to the following categories:

a.) Definite: Adverse event is clearly related to investigational procedures(s)/agent(s).

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

4. Plan for Grading Adverse Events:

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

1. Mild adverse event
2. Moderate adverse event
3. Severe adverse event

5. Plan for Determining Seriousness of Adverse Events:

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

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

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

6. Plan for reporting serious AND unanticipated AND related adverse events anticipated adverse events occurring at a greater frequency than expected, and other unanticipated problems involving risks to subjects or others to the HIC

The investigator will report the following types of adverse events to the VA HSS and Yale HIC:

a) Serious AND unanticipated AND possibly, probably or definitely related events;
b) Anticipated adverse events occurring with a greater frequency than expected; and
c) Other unanticipated problems involving risks to subjects or others.

These adverse events or unanticipated problems involving risks to subjects or others will be reported to the HIC or HSC within 5 calendar days of it becoming known to the investigator, using the appropriate forms found on the website.

7. Plan for reporting adverse events to co-investigators on the study, funding and

regulatory agencies

For the current study, the following individuals, funding, and/or regulatory agencies will be notified:

☐ All Co-Investigators listed on the protocol.
☐ Brain & Behavior Research Foundation NARSAD Young Investigator Grants.

The principal investigator, Gihyun Yoon, M.D., will conduct a review of all adverse events upon completion of every study subject. He will evaluate the frequency and severity of the adverse events and determine if modifications to the protocol or consent form are required.

5. Payments for Participation

In this pilot project, subjects will be compensated for their participation. Payment for participation will be given in the form of cash at the time of their participation. Subject will be paid $100 for each MR study visit (second and third), $150 for the final MR study visit (fifth), and $50 for each non-MR study visit (first and fourth). Therefore, we anticipate the subject's total earning – if he/she participates in all sessions is $450. At the principal investigator's discretion, further subject payment may be made for travel, parking, lodging, food or transportation.

6. Data Collection
Study data will be collected and managed using REDCap electronic data capture tools. REDCap (Research Electronic Data Capture) is a secure, web-based application designed to support data capture for research studies, providing 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for importing data from external sources.

Reference: Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. Journal of biomedical informatics. 2009;42:377-381.

7. Data Analysis
A sample size of 20 participants, in this pilot project, will be analyzed to detect meaningful differences in the outcome variables. When the number of subjects is 20 in this double-blind, randomized, crossover study, an analysis of variance will have at least 80% power to detect difference in means for the intranasal insulin vs. placebo (significance level: 0.05)—based on other intranasal insulin studies [31, 43] We will use a mixed model ANOVA to statistically analyze the psychiatric assessments and fMRI data (with patients as a random variable and treatment condition [intranasal insulin or placebo] as a within-subjects variable).
The primary outcome measure will be the CAPS and the secondary outcome measures will be the CGI, HAM-A, and PCL. For fMRI data, BOLD responses will be analyzed at the whole-brain level and using ROIs in the amygdala, anterior hippocampus, and medial prefrontal cortex based on published coordinates. Standard image processing and analyses will be conducted on the fMRI data, including structural and functional analyses. Exploratory examination of correlations between fMRI measures and behavioral measures will use standard Pearson's correlational analysis.

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