D-Cycloserine for the Treatment of Chronic, Refractory Low Back Pain

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Version Number: 5.0

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STATEMENT OF COMPLIANCE

The study will be conducted in accordance with the International Conference on Harmonisation guidelines for Good Clinical Practice (ICH E6), the Code of Federal Regulations on the Protection of Human Participants (45 CFR Part 46), and the DOD Terms of Award. All personnel involved in the conduct of this study have completed human subjects protection training.

SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH guidelines.

Principal Investigator:

Signed: _____ Date: _____

Name: Thomas J. Schnitzer, MD, PhD

Title: Professor, Department of Physical Medicine and Rehabilitation

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LIST OF ABBREVIATIONS

ACS	Attentional Control Scale
AE	Adverse Event/Adverse Experience
BDI	Beck Depression Inventory
BID	Twice Daily
BP	Blood Pressure
CBC	Complete Blood Count
CBP	Chronic Back Pain
CFR	Code Of Federal Regulations
CNS	Central Nervous System
CONSORT	Consolidated Standards Of Reporting Trials
CPAQ	Chronic Pain Acceptance Questionnaire
CRF	Case Report Form
DHHS	Department Of Health And Human Services
DTI	Diffusion Tensor Imaging
eACS	Emotional Attentional Control Scale
eCRF	Electronic Case Report Form
ERQ	Emotional Regulation Questionnaire
FDA	Food And Drug Administration
FFMQ	Five Facet Mindfulness Questionnaire
fMRI	Functional Magnetic Resonance Imaging
g/dL	Gram Per Deciliter
ICF	Informed Consent Form
ICH	International Conference On Harmonisation
IND	Investigational New Drug Application
IP	Investigational Product
IRB	Institutional Review Board
ISM	Independent Safety Monitor
IUD	Intrauterine Device
LAQ	Loss Aversion Questionnaire
LOT-R	Life Orientation Test (Revised)
MAIA	Multidimensional Assessment of Interoceptive Awareness
mg	Milligram

mL	Milliliter
MMRM	Mixed Model Repeated Measure
mPFC	Medial Prefrontal Cortex
MPQ	McGill Pain Questionnaire
MRI	Magnetic Resonance Imaging
N	Number (Typically Refers To Participants)
NAc	Nucleus Accumbens
NEO-FFI	Neuroticism-Extraversion-Openness Five-Factor Inventory
NIH	National Institutes Of Health
NMFF	Northwestern Medical Faculty Foundation
NMH	Northwestern Memorial Hospital
NMPG	Northwestern Memorial Physicians Group
NRS	Numerical Rating Scale
NSAID	Non-Steroidal Anti-Inflammatory Drug
NU	Northwestern University
NUCATS	Northwestern University Clinical and Translational Sciences Institute
ODI	Oswestry Disability Index
PANAS	Positive And Negative Affect Schedule
PASS-20	Pain Anxiety Symptoms Scale
PCS	Pain Catastrophizing Scale
PDQ	painDETECT Questionnaire
PFc	Prefrontal Cortex
pg	Picogram
PGA	Patient Global Assessment
PGIC	Patient Global Impression of Change
PHI	Protected Health Information
PI	Principal Investigator
PRO	Patient Reported Outcome
PSQ	Pain Sensitivity Questionnaire
QD	Once Daily
SAE	Serious Adverse Event/Serious Adverse Experience
SBP	Subacute Back Pain
SBPp	Subacute Back Pain – Persisting Phenotype

SBPr	Subacute Back Pain – Recovering Phenotype
SGOT	Serum Glutamic Oxaloacetic Transaminase
SGPT	Serum Glutamic Pyruvic Transaminase
SNRI	Serotonin-Norepinephrine Reuptake Inhibitor
SOP	Standard Operating Procedure
SSRI	Selective Serotonin Reuptake Inhibitor
TID	Thrice Daily
Unit/L	Unit Per Liter
US	United States
WOCBP	Women Of Childbearing Potential

PROTOCOL SUMMARY

Title: ***D-Cycloserine for the Treatment of Chronic, Refractory Low Back Pain***

Summary: This study is a 26-week, double-blind, randomized, placebo-controlled two-arm parallel-group trial of d-cycloserine, pharmacological treatment selected based on positive results from previous preclinical and clinical studies, for the treatment of **chronic, refractory low back pain (CBP).** After a 2-week screening period, individuals will be randomized to receive either 12 weeks of d-cycloserine or placebo and then followed for an additional 12 weeks to evaluate persistence of benefit at study endpoint, 24 weeks after randomization. During the 12-week treatment period, participants will undergo evaluation at baseline and at clinic visits on weeks 2, 6 and 12 after randomization to assess pain, proper treatment use and side effects. During the subsequent 12-week follow-up period, pain and safety will continue to be assessed monthly by phone calls. All patients will also be assessed daily using an electronic diary (eDiary) to record pain and mood. T1-MRI, resting state fMRI DTI-MRI, and ASL will be performed at baseline and at the end of 12 weeks for individuals completing MRI.

Objectives: Primary: To determine if treatment with d-cycloserine in individuals with chronic, refractory low back pain will demonstrate greater reduction in pain compared to individuals treated with placebo.

Secondary: To determine whether treatment efficacy is gender dependent.

Secondary: To determine the brain reorganization with treatment response with identification of biomarkers to predict response to treatment

Secondary: To develop a self-report measurement tool that will predict response to treatment

Population: Sample size: 244
Gender: Male and Female
Age: 18 years and older
Demographic group: No racial/ethnic restrictions
General health status: History of low back pain for a minimum of 6 months and good general health.

Phase:	2
Number of Sites:	One Northwestern University Chicago, IL 60611
Description of Intervention:	Cycloserine is a white to off-white powder that is soluble in water and stable in alkaline solution. It will be available as capsules of 200 mg with matching placebo capsules. Eligible participants will be randomized to either d-cycloserine or placebo after a 1-2 week run-in period. Study medication will be taken twice a day (BID) for 12 weeks.
Study Duration:	48 months
Participant Participation Duration:	26 weeks
Estimated Time to Complete Enrollment:	42 months

1. KEY ROLES AND CONTACT INFORMATION

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2. INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Chronic pain has major personal, financial, and societal consequences

Pain is the primary reason why people seek healthcare. Everyday millions of Americans are either partially or totally disabled by pain. If untreated, this pain can lead to depression, insomnia, depressed immune function, changes in eating patterns, impaired cognitive function, and other long-term deleterious effects commonly associated with chronic pain.[1]

When pain is the consequence of an acute injury and/or inflammatory process, it can often be alleviated through attenuation of the noxious stimulus or disease processes driving the inflammation. Most acute pain conditions are managed with simple analgesics, anti-inflammatory drugs and/or opiate medications. However, pain can become chronic in nature. Despite a considerable research effort, there is no generally effective therapy for chronic pain. This condition affects at least 116 million American adults - more than the total affected by heart disease, cancer, and diabetes combined. Chronic pain also costs the US over half a trillion dollars each year in medical treatment and lost productivity [1]. A World Health Organization report indicates that chronic pain is the top source of disability in the US and the 6th largest source of disability worldwide [2]. Epidemiological studies show that 1 in 5 individuals suffer from chronic pain, and this rate is increasing [3-9]. Most often, chronic pain persists for a lifetime. Thus, chronic pain remains an enormous medical and societal burden.

Chronic low back pain is a common and impactful societal and military problem

Chronic low back pain constitutes the major form of chronic pain, with a prevalence as high as 70-85% in adults at some time in their lives. [10] In the US, low back pain is the most common cause of activity limitation in adults younger than 45 years of age and the second most frequent reason for physician visits [1, 11]. Estimates from other countries support the fact that low back pain is one of the major, if not the major, cause of days lost from work and results in significant costs to both the economy and health care system.[2]

While it is well recognized that back pain is prevalent in the civilian population, several studies have highlighted its particular significance for the military, including the active military. “It might not be the first thing that comes to mind when you think of wounded warriors, but back pain is a prevalent problem that leads to a large number of evacuations downrange. Specifically, musculoskeletal pain and spinal pain syndromes are the most common cause, accounting for between 30 to 35 percent of evacuations form Iraq and Afghanistan.” [12] In a study of active military,[12] using the US Defense Medical Epidemiology Database, the incidence of low back pain was reported as 4.05 per 100 patient years, meaning 4 people out of each 100 per year. The incidence was significantly higher in both those <20 years and >40 years of age, and almost twice as high in the

Army compared to Marines, with rates approaching 1 out of 10 individuals affected per year. Many of the long-term spine casualties in the deployed military are not due to direct trauma. "The research reports that there have been 10 times as many long-term spinal pain casualties unrelated to the physical injuries of battle reported in these recent conflicts…Soldiers who left combat areas with low back pain complaints had just a 13.3 percent chance of returning to their units." [12]

Importantly, epidemiologic studies have identified that non-battle injuries are now the major cause of service member attrition during combat, and that the prevalence of low back pain among those engaged in heavy physical training may be more the "rule" than the "exception". [13] In a 5-year prospective cohort study of US Army Special Operations reserve officers, in those who reported on back pain for the previous 3 years, over the subsequent 18 months of follow-up 64% reported moderate back pain and 84% reported mild back pain.[14] There is a high prevalence of chronic back pain reported not only in military personnel deployed in combat zones but also in those who are not. Thus, among American Gulf War veterans, the most common healthcare problem was back pain (17%),[15] followed by joint pain (15%) whereas in British Gulf veterans back pain again was the most commonly reported medical problem, reported in 36%.[16] The latter study also evaluated a similar group of military deployed to a non-war zone (Bosnia) and a control military group deployed to neither (Era), and back pain was again the major reported health issue (Bosnia, 24%; Era, 28%). Thus, back pain is common in all stages of military service and life and represents an important healthcare concern for active military and for veterans.

No effective treatment exists for chronic back pain

Despite a long list of available pharmacological and management strategies, a significant percentage of patients with chronic back pain (CBP) remain dissatisfied with their levels of pain relief. Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most widely used treatments for acute back pain, for which there are data demonstrating modest effectiveness. A number of reviews of existing randomized clinical trials in back pain have been published over the last decade with an initial Cochrane Collaboration review in 2008 which was updated in 2016.[17] Most remarkable about the conclusions of this most recent review regarding NSAIDs is the truly modest effectiveness of NSAIDs (a mean improvement of 3.3 points on a 0-100 visual analog scale). Studies of opioids have demonstrated similar results, with efficacy in short-term studies that is limited and similar to NSAIDs.[18, 19] There are few high-quality studies of other treatment modalities in CBP, and those that exist with agents such as duloxetine [20, 21] demonstrate again modest efficacy over short period of time. Thus, despite numerous available pharmacological and management strategies, what is evident is that a significant percentage of CBP patients remain dissatisfied with their levels of pain relief, and the World Health Organization has concluded that no available treatments are superior to others [22].

More important than their modest efficacy when used acutely and the total lack of long-term data regarding efficacy when used chronically are the known serious and life-threatening side effects of these medications. The NSAIDs, as a class, carry a boxed warning from the FDA of two potential fatal risks in their prescribing information, one regarding the risk of increased life-threatening cardiovascular events and the other for the possibility of gastro-intestinal bleeding with potential mortality. Opioids have protean adverse effects associated with their use, ranging from constipation, decreased mental acuity, dizziness and increased falls and fractures to the well-recognized risk for abuse and addiction.[23] Other centrally acting agents, such as duloxetine, have a significantly increased risk of side effects.[20, 21] Thus, there is an urgent need to develop novel pain management options especially for CBP.

2.2. Rationale

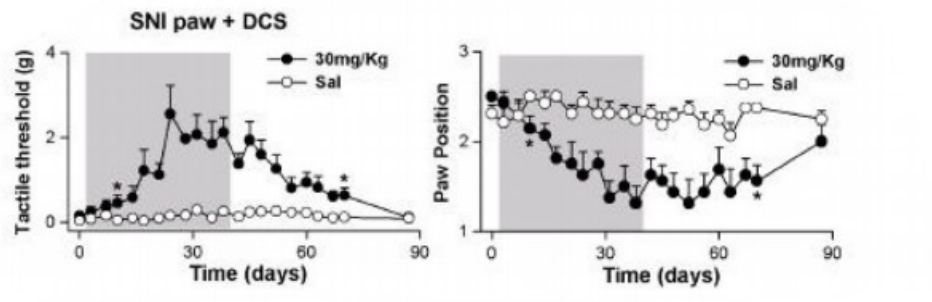
D-Cycloserine as a therapeutic agent

D-cycloserine (DCS) is an established, FDA-approved antimicrobial agent, recommended to be used at doses up to 1000mg/day for the treatment of tuberculosis in the United States and widely used throughout the world as a second-line agent for that disease.{, #1609@@hidden} More recently, it has been shown to have glycinergic activity in the brain. It is an excitatory amino acid and partial agonist at the glycine binding site of the NMDA receptor. [24] At low doses, it enhances learning and memory. At high-doses, it is utilized as an anti-convulsant.[25]. DCS is reported to relieve symptoms of opioid withdrawal,[26] and it has also been tested in a number of neuropsychiatric disorders, with positive results in conditions including schizophrenia and autism[27-29] but lack of efficacy in others, such as Alzheimer's disease.[30]

D-Cycloserine in chronic pain

In rodents, oral DCS has been shown by our group to decrease pain-like behaviors in multiple models of chronic pain (spared nerve injury, chemotherapy induced neuropathy), in a dose-dependent manner, with efficacy increasing with longer duration use [31]. Moreover, animals already treated with DCS, when re-treated showed greater magnitude and longer duration alleviation of neuropathic

Fig. 1. Extended treatment with DCS results in larger decreases in pain behavior, which were sustained after cessation of treatment. Rats were treated for 39 days, and tested twice per week during treatment and for 49 days after treatment.



pain, and when treated for about 30 days, post-treatment alleviation of symptoms persisted for more than an additional 30 days (Fig. 1) [31]. Although at the doses tested DCS in neuropathic rats relieved pain-like symptoms by only about 50%, the emotional impact of the neuropathic condition seemed completely eliminated (i.e., animals behaved as if the remaining pain does not bother them). It seems that in neuropathic rodents DCS does not change stimulus sensitivity for body parts outside of the injury, and acute or single doses of DCS do not alleviate neuropathic pain. Therefore, in the rodent DCS does not act as a short-term analgesic; instead, it reduces neuropathic pain and seems to largely eliminate associated negative affect.

The initial study of DCS in the rat described above was in fact a reverse translational effort, as it was based on the evidence that previous brain imaging studies in CBP patients had shown that chronic pain preferentially activates the medial prefrontal cortex (mPFC) and the amygdala, [32] that DCS administration systemically or centrally (within mPFC or amygdala) in the rodent facilitates fear extinction, [33] and that the mPFC is critical in the rodent extinction of fear [34]. More recent studies provide strong additional evidence regarding the role of the mPFC in human chronic pain [35-39] and its interaction with subcortical limbic structures most notably the hippocampus, amygdala and nucleus accumbens [37-40] as well as rodent evidence showing that direct manipulation of components of this circuitry either disrupts or modulates persistent pain behavior [41-44]. We have hypothesized that chronification of pain critically depends on brain memory circuitry[45] and DCS enhances cognitive and memory processes, and especially fear extinction, [46-48] through prefrontal limbic circuitry and reduces persistent pain in the rodent [31].

Proof of concept pilot trial

Based on the demonstrated efficacy of DCS in animal studies of chronic pain as noted above, we initiated a small, proof-of-concept pilot study of DCS in people with chronic low back pain. We utilized a randomized, parallel-group design having a placebo control with the goal of being able to determine if there was any evidence of efficacy, and if so, to define an effect size of DCS for pain relief so as to provide information for a future, adequately powered, definitive study.

Forty-one participants were enrolled; all met fairly standard inclusion and exclusion criteria (between ages of 18 and 75 years, males and females, back pain for at least 6 months, pain level of ≥5 on a 0-10 NRS pain scale, no inflammatory arthropathy or fibromyalgia; see NCT00125528 on clinicaltrials.gov and published manuscript [49]). After screening, participants discontinued their pain medications and were randomized to either DCS or placebo. Treatment was started with a dose of 50mg DCS bid or matching placebo and escalated each 2 weeks, first to 100mg bid and then 200mg bid or matching placebo. After 6 week, the group receiving DCS had a greater reduction in pain than the placebo group, with an effect size of 0.4. The difference was 1.0 unit on the NRS scale, which is clinically meaningful. (Fig. 2) This difference did not reach statistical significance (p=0.14), due largely to the limited sample size of the study. However, all the study efficacy endpoints showed DCS to be more beneficial than placebo We were aware that the study would more than likely be underpowered but wanted to be able to determine an effect size to inform future studies.

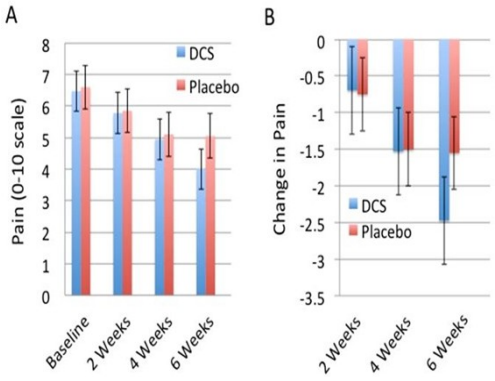


Fig. 2. A. Absolute NRS pain level at baseline and each subsequent study visit. B. Change in pain from baseline. Mean values±SE.

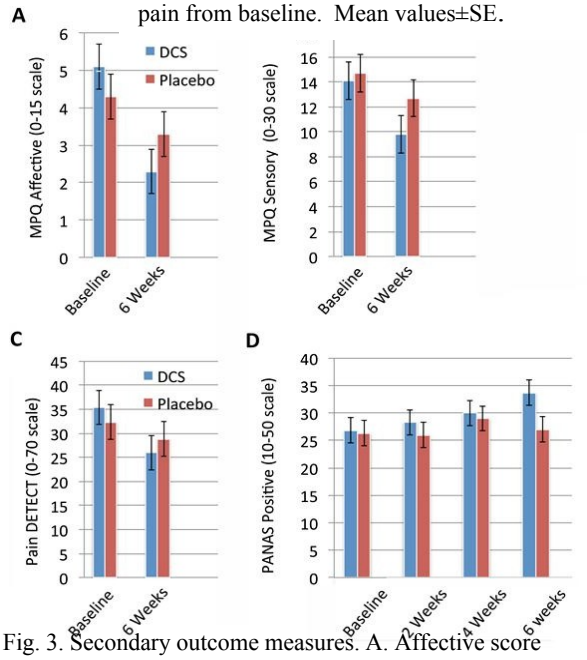


Fig. 3. Secondary outcome measures. A. Affective score of McGill Pain Questionnaire. B. sf-MPQ sensory score. C. Pain DETECT. D. PANAS positive affect score. All measures indicate a trend or significant improvement with DCS in comparison to placebo. Error bars are SEMs.

In addition to the NRS pain scale, we also evaluated a number of secondary endpoints, with a particular focus on those that would provide information about changes in the affective component of participants' existing pain, based again on the earlier animal studies. What we observed were borderline significant time x treatment interactions for MPQ affective and sensory, the painDETECT and PANAS positive (Fig. 3). The increase in PANAS positive scores at 6 weeks of DCS treatment (with no change in PANAS negative scores), suggests improvement in positive emotional affect, reflecting higher energy, more pleasurable engagement, and increased extroversion. Thus, improved mood accompanied with decreased pain seem to also have been observed in the chronic low back pain participants receiving DCS.

Based on these outcomes we are therefore proposing in this submission a larger phase 2 study of DCS in people with chronic low back pain to establish its efficacy in this condition. An effect size of 0.4 is in the range between NSAIDs and opioids, but most importantly DCS was not associated with any of the serious side effects of either of those two classes of drugs, thus allowing it to be widely and safely used, replacing those agents, if it were to be approved for this indication.

Brain biomarkers of pain chronicity and response to treatment

We have also recently demonstrated the ability to use brain imaging to predict long-term pain response and plan to do brain imaging at baseline to validate these findings in the current population.[37, 39] Additionally, brain imaging at end of the treatment period will permit definition of brain biomarkers that could help us to better understand the brain processes involved in those who respond as well as those who do not, providing a basis for the development of a tool for clinicians to predict responders and non-responders (personalized medicine). Moreover comparing brain anatomy and function before and after treatment between responders and non-responders will provide objective measures of treatment efficacy

Chronic pain is a state of continued negative emotional suffering, and the limbic brain had been originally hypothesized[50, 51] and now demonstrated by results of natural injury "experiments" [52]and more recently neuroimaging studies in man and animals from our group and others (reviewed in [53]) to be involved in chronic pain. The main brain regions comprising the limbic system are the prefrontal cortex (medial, mPFC, and orbital, OFC), nucleus accumbens (NAc), hippocampus, and amygdala. Recent data provides converging evidence that components of this circuitry are important for chronic pain. In humans, the NAc encodes salience of impending pain and the reward value of analgesia, which is distorted in chronic back pain (CBP) [54] and functional connectivity between NAc and mPFC prospectively predicts chronification of pain. [37]. Prefrontal cortical activity encodes subjective reports of back pain intensity in CBP patients, [32, 38] and this region is assumed to regulate the meaning of affective responses and to influence emotional decision-making. Meanwhile, the size of the amygdala and hippocampus shows strong genetic dependences [55] and both structures exhibit changes in activity and functional connectivity during the transition to chronic pain. [38, 40] Recent studies from our group have demonstrated that limbic white matter connections predisposed patients to chronic pain. Intra-limbic white matter connectivity analysis identified three segregated communities: mPFC-amygdala-accumbens, OFC-amygdala, and OFC-amygdala-hippocampus. Higher incidence of white matter and functional connections within the mPFC-amygdala-accumbens circuit, as well as smaller amygdala volume, represented independent risk

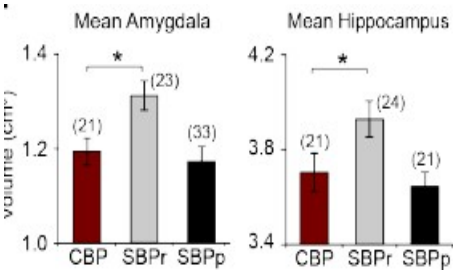


Fig. 4. Amygdala and hippocampal volumes in age-matched CBP subjects. Significantly smaller than individuals who had subacute back pain and recovered (SBPr) $p = 0.009$, $p = 0.05$, respectively and comparable to those with subacute back pain but did not recover (SBPp))

factors for chronic pain.[53] Additionally, examination of candidate genes revealed opioid receptor-mediated neural susceptibility to chronic pain.[53]

Brain imaging can also provide an objective measure of pain response. We had shown previously that brain anatomy undergoes regional decreases in grey matter density with the development of chronic pain[56], and other groups have demonstrated that relief of pain can result in renormalization of brain anatomy.[57] In addition we have evidence that the brain functional properties change in chronic pain and that there is a global disruption of information sharing in the brain of CBP patients[58] (figure 5). The extent of this disruption, value of Kd, is directly proportional to the intensity of chronic pain. Therefore, an objective measure of pain relief would be demonstrating that Kd decreases with treatment.

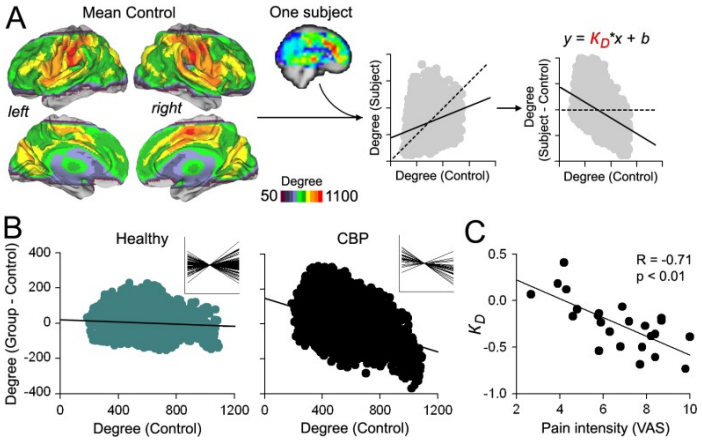


Figure 5. Brain connectome disruption identifies chronic pain. **(A)** Method for deriving k_D: Mean degree map from off-site healthy controls (n = 129, C1000). For any given subject, the mean degree of each node in the off-site healthy control group (x axis) is plotted versus degree value for that node in a subject. Instead for each node degree value, if we plot the difference in degree (subject – off-site control) across all nodes, then the slope defines k_D. **(B)** Scatter plots depict group k_D for our healthy (n=70, green) and CBP patients (n=25, black) compared to the off-site healthy control group. Inserts show individual k_D value

We have recently defined and validated specific brain biomarkers for both response to placebo as well as response to duloxetine in individuals with chronic pain.[59] Two studies were performed, an initial study to identify and define one or more biomarkers of response to placebo and a subsequent, independent trial to act as validation of this biomarker response with regard to placebo as well as to define response to an active comparator, duloxetine. In the first discovery study, right mid-frontal gyrus (r-MFG) connectivity was found to be the brain region that best identified placebo responders (Fig. 6, below). In the subsequent study, the same measure prospectively identified placebo responders (95% correct), and predicted the magnitude of the placebo response. Therefore, r-MFG connectivity represents a neural marker for placebo response in the clinical trial setting in OA.

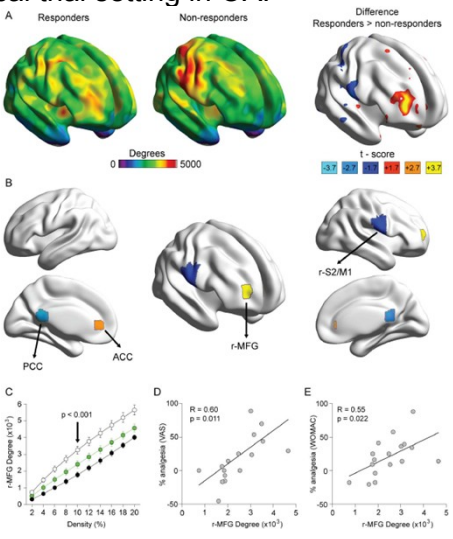


Fig. 6. Patterns of brain connectivity in responders and non-responders to placebo. (A) Average brain maps for degree count (number of connections to any location in the rest of the brain) in responders and non-responders. (b) Brain regions highlighted were identified based on minimal t-score and threshold-free cluster enhancement. Right mid-frontal gyrus (r-MFG) was the region with the highest significance between group difference, while bilateral anterior cingulate (ACC), posterior cingulate cortex (QPCC), and region overlapping the right secondary somatosensory and primary motor cortex (r-S2.M1) had lower significance differences. (C) Degree counts derived from r-MFG region in OA patients classified as responders

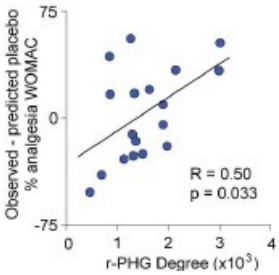


Fig. 7. r-PHG degree counts correlated with pain outcomes

In addition to evaluating brain regions associated with the response to placebo, we also closely examined correlations of brain biomarkers and response to duloxetine in the second study. This led to the discovery that the degree of right parahippocampal connectivity predicted the magnitude of the response to duloxetine analgesia[59] (Fig. 7). Thus, our evidence is consistent with clinical treatment responses, either to placebo or to active drugs, having biological underpinnings in the brain and paves the way to the development of a clinical instrument (Aim 3) that can provide utility in the pain management setting and lead to evidence-based individualized treatment decision-making.

3. OBJECTIVES

Because of the lack of effective and safe treatments for chronic pain, and the prevalence and impact of chronic pain on individuals, society and the military, a non-opioid alternative to current treatments that is both safe and effective at alleviating pain and symptoms is badly needed and would represent a major advance in pain management. This proposal focuses on one agent, DCS, for which there are preliminary data in humans for efficacy and safety, and further extends the original pilot study with imaging technology to identify brain biomarkers predictive of response to therapy which will allow the development of a clinical instrument to guide decisions regarding patients' pain management. Thus, this study will provide evidence for not only a new, effective and safer medication for pain, but also a means to provide more individualized treatment based on the use of biomarkers and the development of such a clinical instrument.

Specific Aim 1. Determine the efficacy and safety of DCS compared to placebo to reduce pain in people with chronic low back pain

Hypothesis: The group of people receiving DCS will report lower pain ratings than those receiving placebo.

Approach: An adequately powered, double-blind, parallel-group, randomized trial of DCS vs placebo will be undertaken in people with CBP. This study will be of 24 weeks' duration, and outcome measures will include not only magnitude of pain

relief but also changes in behavior and affect. For this aim pain relief will be documented using standard subjective reports.

Specific Aim 2. Define brain biomarkers that will allow prediction of people who will respond to specific intervention, placebo or DCS, in this population

Hypothesis: Anatomic and functional brain biomarkers will be defined that will differentiate pain responders from non-responders; a set of these biomarkers will predict pain response. A separate set of brain biomarkers will identify pain relief based on functional and anatomical reorganization of the brain, thereby demonstrating treatment efficacy based on objective outcomes.

Approach: Anatomic and functional brain MR imaging will be performed before and after treatment with DCS or placebo, and brain biomarkers analysis will be undertaken as previously described.[37, 39, 49, 59, 60]

Specific Aim 3. Develop a self-report measurement tool to predict the probability of CBP patients responding to DCS and/or placebo.

Hypothesis: The probability of response to treatment will be predicted based on self-report measures determined by individuals with CPB.

Approach: Multiple questionnaires of participants receiving placebo and DCS will be used to identify a set of scales that correlate with brain imaging biomarkers for pain response in CBP. A refined, validated set of these items will constitute the tool for Likelihood of Therapeutic Response (LTR).

4. STUDY DESIGN

Aim 1: Determine the efficacy and safety of DCS compared to placebo to reduce pain in people with chronic low back pain

We are proposing an appropriately powered, 24 week, double-blind, placebo-controlled trial of DCS compared to placebo in individuals with chronic low back pain (Fig. 8).

Study design overview

Potential participants will undergo an initial screening visit to determine eligibility and interest. Those who qualify (≥18 years of age, either sex, pain level of ≥4 on a 0-10 NRS pain scale; see full list of inclusion and exclusion criteria below) will be asked to record pain levels twice daily in an eDiary that is accessible via a smartphone device or computer, discontinue medications used to treat their CBP, and return for baseline visit in 10-14 days. They will be given acetaminophen to be used if required for back pain, up to 3000 mg/day. If they maintain a mean pain level of ≥4 and ≤9 over the 5-7 days (minimum of daily eDiary entries for at least 5 of 7 days) prior to their baseline visit and have a pain level ≥4 at baseline, they will be eligible to be randomized. They will first be asked to complete a battery of questionnaires (NRS pain scale, Patient Global Assessment, Patient Global Impression of Change, McGill Pain Questionnaire, NIH PROMIS Profile, PainDETECT, Positive and Negative Affect Schedule, Beck Depression Inventory, Pain Catastrophizing Scale, Multidimensional Assessment of Interoceptive Awareness, Oswestry Disability Index, SF-12 Health Survey, Chronic Pain Acceptance Questionnaire, Pain Anxiety Symptoms Scale, Pain Sensitivity Questionnaire, Emotional Regulation Questionnaire, Attentional Control Scale, Emotional Attentional Control Scale, Five Facet Mindfulness Questionnaire, Neuroticism-Extraversion-Openness (NEO) Five Factor Inventory, Life Orientation Test (Revised), and Loss Aversion Questionnaire) and will then undergo brain imaging (MRI). After completion of these procedures, participants will then be randomized to either DCS 200mg or placebo in identical capsules, one

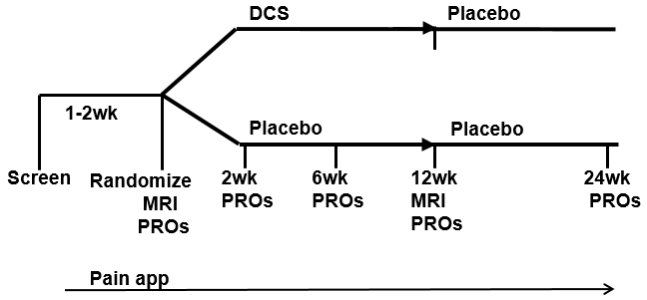


Fig. 8. Study design

capsule to be taken twice daily (see section, Intervention, for additional details on dose and dosing regimen for DCS) and asked to continue to record pain levels twice daily as well as indicate study drug compliance. This eDiary technology has been utilized successfully in a number of our previous longitudinal pain trials. Participants will then be seen again for follow-up visits at 2 weeks, 6 weeks, 12 weeks and 24 weeks after randomization. At each visit they will complete questionnaires to determine pain magnitude and characteristics, have vital signs checked, and safety data collected.

Additional study medication will be dispensed at the 2 week and 6 week visits. At the 12 week visit, the same information will be collected and another brain MRI performed. Placebo will be dispensed at this time in order to evaluate persistence of the benefit of treatment over the following 12 weeks. Participants will continue to report pain via the eDiary and will be given rescue acetaminophen.

4.1. Treatment Assignment Procedures: Randomization and Masking

Randomization Procedures

Participants will be assigned to treatment arms (DCS vs placebo) based on a computer generated permuted block randomization with block size randomly varied and an allocation ratio of 1:1. Allocation concealment will be ensured by utilization of sequentially numbered containers. A designated unblinded individual will be responsible for assuring proper medication assignment to each container. The randomization code will be maintained by the designated unblinded individual and will be available in cases of emergency or clinical situations in which knowing the treatment allocation would make a difference in the safety or management of a subject. In such a circumstance, the allocation assignment will be made available after consultation with the site investigator and the principal investigator. At study conclusion, after database lock, the randomization code will be made available to the study statistician and other personnel analyzing the data.

Masking Procedures

Masking will be achieved by utilizing identical opaque capsules for both DCS and placebo. As described in the Intervention attachment, DCS capsules and identical matching placebo will be provided by the manufacturer. These will be shipped to the research center in bulk, and the only person who will know which capsules contain active vs placebo will be the designated unblinded individual responsible for filling the numbered medication bottles with the appropriate kind and number of capsules. This person will have no involvement in clinical assessments of study subjects. Unmasking should not be required during the study. If allocation assignment needs to be made available, a separate copy of the allocation assignments will be maintained by the designated unblinded individual and made available to the principal investigator for documented emergencies.

4.2. Outcome Measures (see section 6.4 Surveys and Questionnaires for a further description of these instruments)

Primary outcome measure

The primary outcome measure for efficacy is the NRS pain score. Mean pain levels will be assessed at study baseline (mean pain over the 5 days prior to randomization) and compared to mean pain levels for the 5 days prior to the visit at week 12 (study efficacy endpoint). Pain will be assessed using an 11-point NRS scale.

Secondary outcome measures

Durability of pain response will be evaluated by assessing pain response after DCS therapy has been discontinued in double-blinded fashion between weeks 12 and 24. In addition, a number of other secondary outcome measures will be evaluated (see below). Many of these will add information regarding the response to treatment in this study. Specifically, analyses will be done utilizing not only endpoint data but pain (and other) data collected at each of the clinic visits. This will permit further analyses of the pain data which will complement and extend the primary endpoint analysis. Other pain-associated parameters will also be evaluated as covariates in regard to the basic pain outcome. Additionally, brain parameters obtained by fMRI will be assessed and correlated with the clinical outcome measures. Safety will also be assessed as a secondary outcome measure.

A list of major secondary outcome measures includes:

1. Effect of gender on magnitude of pain response.
2. Brain biomarkers (obtained at MRI visits)
3. Patient Global Assessment
4. Patient Global Impression of Change
5. McGill Pain Questionnaire (obtained at Screening, Week 12, and Week 24)
6. painDETECT instrument (obtained at Screening, Week 12, and Week 24)
7. Beck Depression Inventory – Second version (obtained at Screening, Week 12, and Week 24)
8. Positive and Negative Affect Schedule (obtained at Screening, Week 12, and Week 24)
9. Pain Catastrophizing Scale (obtained at Screening, Week 12, and Week 24)
10. Multidimensional Assessment of Interoceptive Awareness (obtained at Screening, Week 12, and Week 24)
11. Oswestry Disability Index (obtained at Screening, Week 12, and Week 24)
12. SF-12 Health Survey (obtained at Screening, Week 12, and Week 24)
13. Chronic Pain Acceptance Questionnaire (obtained at Screening and Week 24)
14. Pain Anxiety Symptoms Scale (obtained at Screening and Week 24)
15. Pain Sensitivity Questionnaire (obtained at Screening and Week 24)
16. Emotional Regulation Questionnaire (obtained at Screening and Week 24)
17. Attentional Control Scale (obtained at Screening and Week 24)
18. Emotional Attentional Control Scale (obtained at Screening and Week 24)
19. Five Facet Mindfulness Questionnaire (obtained at Screening and Week 24)

20. Neuroticism-Extraversion-Openess (NEO) Five Factor Inventory (obtained at Screening and Week 24)
21. Life Orientation Test (Revised) (obtained at Screening and Week 24)
22. Loss Aversion Questionnaire (obtained at Screening and Week 24)

- Measure 1 will assess the interaction between the primary endpoint and gender.
- Measure 4 will evaluate the interaction between the primary endpoint and specified brain biomarkers with particular attention to corticostriatal connectivity. There are four predefined measures extracted from these scans: right amygdala volume, white matter fractional anisotropy, mPFC-NAc functional connectivity and mPFC/somatosensory link ratio. Only mPFC-NAc and mPFC/somatosensory link ratio values are expected to reflect treatment effects. Whole-brain exploratory analyses will be used to identify both brain predictors of treatment response and brain reorganization (consequences) in response to treatment. Whole-brain information sharing as defined by Kd reflects intensity of CBP (figure 5). Therefore, changes in Kd can be used as an objective outcome regarding pain relief with treatment. Also regional grey matter density decreases seen in CBP should recover with pain relief. The contrast of this measure between placebo and active treatment before and after study should also provide objective measure for treatment efficacy.
- Measures 5-12 are validated instruments that have been successfully applied to the back pain population.
- Measures 5-6 are expected to reflect the change observed in measure 3, and are thus primarily confirmatory for the main outcome. They will also be used to examine characteristics of the pain that differentiate between treatment responders and non-responders, and this information can be used in future response prediction based studies and in clinical decision making.
- Measures 8-9 are expected to contribute to prediction of treatment response. They will be used as covariates in logistic multiple regression exploratory models where brain parameters, pain parameters and personality values are combined to predict treatment effect.
- Measure 11 will provide information regarding pain impact on various aspects of quality of life and can be used as a confirmatory measure for treatment efficacy.
- Measures 13-22 will be administered to profile psychological factors and personality traits in patients experiencing low back pain

A list of exploratory outcomes includes:

1. Patient-Reported Outcomes Measurement Information System-57 (PROMIS-57)
2. NIH Toolbox

- Measure 1 will be used to assess the reliability and validity of utilizing common PRO metrics to evaluate and monitor physical, mental, and social health specifically in patients with chronic low back pain.
- Measure 2 will be used to longitudinally assess cognitive, emotional, sensory, and motor functions with treatment administration and may function as predictive parameters for treatment response.

4.3. Patient populations

We anticipate screening over 400 individuals (to randomize 244 participants) with chronic back pain over 3 years, all drawn from the Chicago metropolitan area. There are no racial, ethnic or gender restrictions and no upper age limit. It is anticipated that the study population will generally reflect that of Chicago. Our experience from previous chronic back pain studies provides us reassurance that we will be able to identify this number of individuals required to be screened (2-3 people/week) and that we will achieve a diverse participant cohort.

4.4. Recruitment Process

Participants will be drawn from two major pools of individuals: those captured by involvement with the Northwestern Medicine (NM) and Shirley Ryan Ability Lab healthcare systems or with Northwestern University research, and those from the wider Chicago community, with approximately 50% of participants coming from each source.

With regard to those already involved with Northwestern and Shirley Ryan Ability Lab, each maintains an electronic data warehouse (EDW) and patient portal (i.e. MyChart) that contain and make accessible for researchers the electronic medical records of all individuals who are seen by clinicians within the system and who have provided consent to be contacted for research. Access is obtained through requests based on specific ICD-10 codes and providers. All data requests must be approved by an EDW or patient portal data steward for approval. Once the report is received, the patient's physician is contacted to obtain permission to contact them. Letters or electronic messages are then sent out to potential participants who are given the option to either reach out to the study team if they are interested or to let us know that they are not interested. Potential participants are given 10 days to make this decision before we are allowed to reach out by telephone. Data requests are limited to data that are required to conduct the study consistent with the IRB approved protocol. We have used this system since its inception several years ago with great success, allowing us to identify and contact a large number of people with well-defined illnesses, including back pain. We have supplemented this approach by

direct involvement with specific practitioners who deal with large numbers of chronic back pain patients, primarily with the departments of orthopedic surgery, neurosurgery and anesthesiology. We have on-going relationships with specific clinicians and research coordinators within these groups to assist in the identification and recruitment of specific populations. The combination of the EDW referrals and the direct physician referrals assures a high number of potential candidates with well-defined backgrounds and illnesses for studies. We have utilized both these approaches in our past as well as on-going studies in patients with CBP and are confident that these referral sources will continue to be productive. In addition, we will utilize appropriate individuals who are participating in our chronic pain registry (STU00007522). These are individuals with whom we have been in contact and have provided consent to be approached about future clinical studies.

In addition, we will supplement the Northwestern and Shirley Ryan Ability Lab-related pools of individuals with people recruited directly from the Chicago metro area, initially utilizing social media and third party vendors. The study will have its own Facebook page and Facebook advertisements to promote the clinical research study. The Facebook page will promote the study by providing information on the page as well as in the form of IRB-approved posts. These posts will include contact information and a link to a brief pre-screening survey. Additionally, the page will also share publicly available information and news articles regarding low back pain. Any links on the Facebook page will redirect to the pre-screening survey. The pre-screening survey will collect the potential participants' demographic information, brief medical history, and treatment history to determine eligibility for the low back pain study.

Facebook ads promoting the low back pain study will also be published. These ads include information about what the study is for as well as an option to contact study personnel to learn more.

In addition, third party vendors such as ClinicalConnection will be utilized. In those instances, the vendor will post the description of the study provided by our study team on their website. The potential participant will be able to provide their contact information if they wish to do so. All information captured in the process of completing the survey is stored in a database that the website is linked to. It is from this same database the referral information is pulled and issued to sites. Both the website and database are managed by a third party, which the sponsor does not have direct access to. The patient information is only accessible through a link sent directly to the site.

Once the referrals from Facebook and other third party vendor are received, the site will contact the participant via phone in order to complete pre-screening and determine eligibility. The same pre-screening questionnaire will be utilized for all recruitment purposes.

We anticipate a population that will roughly approximate the Chicago area with regard to ethnicity and race (approximately 25% Hispanic; 30% African-American, 40% non-Hispanic White, 5% Asian).

4.5. Retention

As this is a longitudinal study, the ability to have participants return to collect data at multiple time points is crucial to the success of the research. In our previous longitudinal study, an individualized retention program was developed to ensure optimal maintenance of participants throughout the trial. Specific branding of the study (specific name with associated letterhead, literature, etc.), use of low cost, IRB-approved mementos (e.g., calendars, tote bags, etc.), regular communication via email and/or telephone, and recognition of special events (e.g., birthdays, holidays) will be employed to make participants feel engaged. In our experience, the optimal means to enhance retention is repeated contact with the participants, via telephone or mail service or electronically. Our retention rates have also benefited from maximizing flexibility in scheduling study visits and availability of MRI scanning times. We can routinely scan or interview participants any time between 8:00am-9:00pm (including weekends) to accommodate difficult schedules, and missed appointments are typically re-booked within 24 hours. Additionally, the frequency of visits and interactions during the initial 6 weeks of the study when participants come in to obtain their study medication (visits 3-6) will also serve to keep the participants invested in the study and to enhance treatment adherence. These methodologies have been shown to be effective in other longer-term trials and are an important aspect to ensure participants' continued interest and involvement.

4.6. Inclusion and Exclusion Criteria

Participant Inclusion Criteria

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

- Must have a history of low back pain for a minimum of 6 months with or without signs and symptoms of radiculopathy
- Male or female, age 18 years or older, (no racial/ethnic restrictions)
- Must have an average pain score of ≥ 4 (on a 0-10 NRS) over a 5-7 day period (minimum of daily eDiary entries for at least 5 of 7 days) immediately preceding

the baseline visit (visit 2)
- Must be willing to read and able to understand instructions as well as PROs
- Must be in generally stable health
- Must sign an informed consent document after complete explanation of the study documenting that they understand the purpose of the study, procedures to be undertaken, possible benefits, potential risks, and are willing to participate
- Must be willing to discontinue all pain medications for chronic back pain (listed below) except the study medication and rescue medication provided and not use the following prohibited pain medications throughout the duration of the treatment period
    - Analgesics including OTC medications
    - NSAIDs including OTC medications
    - Coxibs
    - Opioids
    - Muscle Relaxants
    - Gabapentinoids including pregabalin and gabapentin
- Must be willing to comply with recording pain, mood, and study treatment adherence twice daily using study eDiary
- Must be willing to abstain from drinking alcohol during the course of the study.
- If female, must be post-menopausal for at least one year or practicing an accepted, highly effective method of contraception or abstinence and plan to continue during the course of the study.

Participant Exclusion Criteria

An individual who meets any of the following criteria will be excluded from participation in this study:

- Low back pain associated with any systemic signs or symptoms, e.g., fever, chills.
- Evidence of rheumatoid arthritis, ankylosing spondylitis, acute vertebral fractures, fibromyalgia or history of surgery or tumor in the back within the past 6 months
- Involvement in litigation regarding their back pain or has a disability claim or is receiving workman's compensation or is seeking either as a result of their low back pain
- Epidural steroid injection within the past 3 months
- History of seizures
- Major new or untreated psychiatric disorder during the past 6 months and/or ongoing treatment with buproprion or fluphenazine
- Beck Depression Inventory II score of >28
- Significant renal disease or severe renal insufficiency
- Substance abuse/dependence including alcohol within the past 6 months
- Significantly abnormal laboratory values
- Pregnant or lactating at the time of randomization
- Known sensitivity to D-cycloserine
- Currently taking any of the following medications: ethionamide, dilantin, isoniazid

(INH)
- In the judgment of the investigator, unable or unwilling to follow the protocol and instructions
- Any change in medication or physical therapy regime for back pain in the last 30 days.
- Chronic progressive neurologic conditions, including Parkinson's disease, Alzheimer's disease, and other conditions associated with dementia
- Other medical disease such as clinically significant congestive heart failure, coronary or peripheral vascular disease, chronic obstructive lung disease, or malignancy
- Presence of undiagnosed skin lesions or history of melanoma
- Current use of recreational drugs
- Current use of medical marijuana
- High dose opioid prophylaxis, defined as > 50mg morphine equivalent/day
- Intra-axial implants (e.g. spinal cord stimulators or pumps)
- Pregnancy or inability to use an effective method of birth control in sexually active men and women while taking the study drug and for one week thereafter. Barrier contraceptives (condoms or diaphragm) with spermicide, intrauterine devices (IUD's), hormonal contraceptives, oral contraceptive pills, surgical sterilization, and complete abstinence are examples of effective methods of contraception.
- Following laboratory abnormalities: liver function tests (SGOT/SGPT) greater than 2.5 times the upper limit of normal; unexplained anemia; evidence of renal insufficiency (creatinine > upper limit of normal) or any other abnormality that the principal investigator feels puts the participant at risk during the study.
- Any medical condition that in the investigator's judgment may prevent the individual from completing the study or put the individual at undue risk
- Lactose allergy
- Ongoing participation in another clinical research study involving an investigational product or having received another investigational product within the last 90 days

4.7. Study Schedule

The study duration is 26 weeks, which includes an initial screening period of up to 4 weeks. There will be six visits during this time: a Screening Visit, Baseline Visit, and four study visits. The schedule for the four study visits after the Baseline Visit will be: Week 2, Week 6, Week 12, and Week 24 (see Fig. 8 above). Treatment with study medication will be terminated after Week 12. Follow-up telephone calls at Week 16 and Week 20 will be made to continue evaluation of participants' back pain until the study end (Week 24). All participants will also use an eDiary to log their back pain intensity, their mood, proper medication use, and other information that they may wish to report. The eDiary data are entered every time participants take their medication (BID) and after termination of treatment (BID) until the final study visit. eDiary entries are immediately transmitted from

a smartphone device or computer through the Internet to a secure web site, downloaded daily to assess compliance, and stored in a second secure server. Our group has been using such an eDiary for about 2 years and they have been very effective. The visit window for all visits will be ± 1 week.

Screening – Visit 1, Week -2 to -4

Screening visit (2-3 hours): Participants will be prescreened over the telephone prior to being invited to attend the screening visit. Those invited will complete the informed consent process and then be evaluated with full inclusion/exclusion criteria. Once consent has been obtained, a medical/pain history and physical examination will be completed. Anthropometric measurements and vital signs will be obtained. Blood will be drawn for laboratory testing, which will include a CBC, chemistry panel and liver function analysis, and urine will be collected for drug screening and pregnancy screen, for women with childbearing potential. Participants will be asked to assess current back pain intensity on a NRS scale (0-10, no pain to worst possible pain; ≥ 4 required to qualify for study). Participants will also complete all PROs electronically and will be instructed on the use of the eDiary. Study staff will ask the participant to discontinue their current pain medications at least 48 hours prior to Visit 2 and to return in 1-4 weeks for the baseline visit. Staff will explain to the participants that only the rescue medication (acetaminophen up to 3000 mg/day) is permitted for pain management during this washout period. If participants return after four weeks for baseline visit, CBC and CMP will be reevaluated.

Questionnaires profiling psychological factors and personality traits will also be completed electronically by participants with the option of doing so during the Screening visit or remotely within three (3) days following the Screening visit.

Baseline visit: MR imaging and randomization – Visit 2, Week 0

Baseline visit (2-3 hours): Participants will complete the NRS pain scale and if their score is ≥ 4 and their average back pain on the eDiary over the last 5-7 days is also ≥ 4 with no changes in clinical status reported, participants will complete PROs electronically, complete the NIH Toolbox assessment, and undergo MRI and fMRI brain scans. Participants who have diabetes will not undergo brain imaging. Vital signs will also be obtained. Participants who meet all entry criteria will be randomized to receive either active treatment or placebo in a double-blind fashion, with sufficient medication dispensed to last until the next visit (including enough for an additional 4 days if needed). For women of child-bearing potential, a urinary pregnancy test will be done prior to receiving study medication. Participants will complete the NRS pain scale and PROs, be asked to continue to record data using the eDiary and to abstain from alcohol.

Interim Visit – Visit 3, Week 2 ± 4 Days
Treatment and safety follow-up (60-90 min). Participants will complete the NRS pain scale, be asked about any changes to their health or concomitant medications, and AEs will be assessed as needed. Vital signs will also be obtained. Treatment compliance will be evaluated by pill counts (participants must take a minimum of 80% of the expected study medication to be considered adherent to study protocol). Study medication (including rescue medication) will be dispensed.

Interim Visit – Visit 4, Week 6 ± 7 Days
Treatment and safety follow-up (60-90 min). All procedures will be performed as described for Visit 3. Participants will be asked about any changes to their health or concomitant medications, and AEs will be assessed as needed. Vital signs will also be obtained. Treatment compliance will be evaluated by pill counts. Study medication (including rescue medication) will be dispensed.

Final on-active treatment Interim Visit – Visit 5, Week 12 ± 7 Days
Treatment and safety follow-up (2-3 hours). All procedures will be performed as described for Visit 2 including MRI scan. Participants who have diabetes will not undergo brain imaging. Participants will be asked about any changes to their health or concomitant medications, and AEs will be assessed as needed. Vital signs will also be obtained. Treatment compliance will be evaluated by pill counts. Study medication (including rescue medication) will be dispensed.

Follow-up Phone Call – Week 16 ± 7 Days
Follow-up phone call (15 min). Participants will be asked if they have had any changes to their health or concomitant medications, and AEs will be assessed as needed. Study staff will record patient-reported study medication use since the previous visit, as well as

the participant's current NRS pain score.

Follow-up Phone Call – Week 20 ± 7 Days
Follow-up phone call (15 min). Participants will be asked if they have had any changes to their health or concomitant medications, and AEs will be assessed as needed. Study staff will record patient-reported study medication use since the previous visit, as well as the participant's current NRS pain score.

Final Study Visit – Visit 6, Week 24 ± 7 Days
Efficacy and safety follow-up (2-3 hours). All procedures will be performed as described for Visit 3. Participants will complete NIH toolbox assessment. Use of study medication will be determined, and participants will be queried about any side effects experienced.

Questionnaires profiling psychological factors and personality traits will also be completed electronically by participants with the option of doing so remotely within three (3) days in advance of or during the Final Study visit.

Interim Electronic Contact

In order to enhance retention of participants in the study and to obtain additional data regarding the course of the participants, participants will be in contact with the site by completing the eDiary on a daily basis. At each of these interactions, the participants will provide an assessment of their level of pain utilizing an 11-point NRS scale.

All participants will be using the eDiary to log pain, mood, and medication use daily. These data are immediately logged into the eDiary server, which provides routine overview of participants' participation and compliance and further enhances retention of participants. If participants repeatedly miss entering these values, they are contacted by phone and instructed for compliance. Participants must submit a minimum of 80% of daily eDiary entries to be considered adherent to study protocol.

Withdrawal Visit
Early termination visit (2-3 hours). If a participant withdraws early or the PI terminates the subject's participation, an early termination visit will be conducted. All procedures will be performed as described for the Final Study Visit (Visit 6). If this occurs before Visit 5, participants will also undergo MRI brain scans with vital signs obtained. All dispensed study medication will be collected and counted. If necessary, study medication to permit taper and discontinuation of study product will be dispensed.

4.8. Study Schedule Schematic

DCS Low Back Pain Protocol										
Study Events	Visit Description	Pre-Screening	Screening	Randomization	Interim Visit	Interim Visit	Efficacy Endpoint	Phone 1	Phone 2	Long-Term Efficacy Endpoint
	Study Visit Number	Visit 0	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5			Visit 6
	Timeline		-2 weeks	Week 0	Week 2	Week 6	Week 12	Week 16	Week 20	Week 24
Standard Evaluations	Screening Eligibility Questions	x	x							
	Informed Consent		x							
	Medical History		x							
	Current Medications/Treatment[2]		x	x	x	x	x	x	x	x
	Current Health Status[2]		x	x	x	x	x	x	x	x
	Vital Signs		x	x	x	x	x			x
	Physical Exam		x							
	Laboratory Assessments		x							
	Urine Pregnancy Test/Drug Screen		x	x[3]			x[3]			
(f)MRI Scans	T1			x[4]			x[4]			
	Resting State Scan			x[4]			x[4]			
	DTI Scan			x[4]			x[4]			
	Arterial Spin Labeling			x[4]			x[4]			
Questionnaires	NRS pain		x	x	x	x	x	x	x	x
	PGIC			x	x	x	x			x
	SF-MPQ		x		x	x	x			x
	PD-Q		x				x			x
	PANAS		x				x			x
	DER		x				x			x
	ODI		x				x			x
	PCS		x				x			x
	WHIM		x				x			x
	SF-12 Health Survey		x				x			x
	CPAQ		x							x
	PASS-20		x							x
	PSQ		x							x
	EMQ		x							x
	ACS		x							x
	EMCS		x							x
	TTMQ		x							x
	NEO-FFI		x							x
	EQ-5R		x							x
	DAQ		x							x
	PROMIS-57			x			x			x
	NIH Toolbox			x			x			x

Important Additional Information	
[1]	This report assesses any changes in treatment or medication throughout the study
[2]	This report assesses any changes to health status and important life events throughout the study (including smoking, drinking, weight, illness, injury, procedures, job changes a
[3]	Urine pregnancy test only will be done on subjects of child-bearing potential at Visits 2 (Randomization) and 5 (Week 12)
[4]	Participants who have diabetes will not undergo brain imaging.

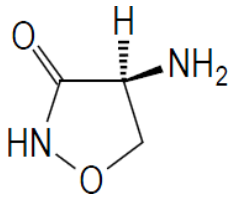
5. STUDY INTERVENTION

5.1. D-cycloserine

a. Key structural and administrative information

(1) Name: D-cycloserine, Seromycin®

(2) Composition: Cycloserine is a white to off-white powder that is soluble in water and stable in alkaline solution. It is rapidly destroyed at a neutral or acid pH. Cycloserine has a pH between 5.5 and 6.5 in a solution containing 100 mg/mL. The molecular weight of cycloserine is 102.09, and it has an empirical formula of C3H6NO2. The structural formula of cycloserine is as follows:



(3) Storage and handling: Study drug will be maintained in a controlled environment between 15^0C-30^0C as required by the product insert.

(4) Source of drug substance: Antibiotice S.A. (Romania)

(5) Dose: 200 mg capsule

(6) Schedule: twice daily

(7) Duration of the intervention: 12 weeks

(8) Concomitant medications allowed: all participants will be allowed to take 1000mg of acetaminophen up to 3 times daily for their pain; current back pain and non-pain medications will be continued

b. Clinical data

(1) Cycloserine is indicated in the treatment of active pulmonary and extrapulmonary tuberculosis (including renal disease) when the causative organisms are susceptible to this drug and when treatment with the primary medications (streptomycin, isoniazid, rifampin, and ethambutol) has proved inadequate. Cycloserine may be effective in the treatment of acute urinary tract infections caused by susceptible strains of gram-positive and gram-negative bacteria, especially Enterobacter spp. and Escherichia coli. It is generally no more and is usually less effective than other antimicrobial agents in the treatment of urinary tract infections caused by bacteria other than mycobacteria.

(2) ADME: After oral administration, cycloserine is readily absorbed from the gastrointestinal tract, with peak blood levels occurring in 4 to 8 hours. Blood levels of 25 to 30 mg/mL can generally be maintained with the usual dosage of 250 mg twice a day, although the relationship of plasma levels to dosage is not always consistent. Concentrations in the cerebrospinal fluid, pleural fluid, fetal blood, and mother's milk approach those found in the serum. Detectable amounts are found in ascitic fluid, bile, sputum, amniotic fluid, and lung and lymph tissues. Approximately 65% of a single dose of cycloserine can be recovered in the urine within 72 hours after oral administration. The remaining 35% is apparently metabolized to unknown substances. The maximum excretion rate occurs 2 to 6 hours after administration, with 50% of the drug eliminated in 12 hours.

(3) Safety of D-cycloserine:

(a) Contra-indicated in people with hypersensitivity to cycloserine

(b) Caution is recommended when used in people with epilepsy, depression, severe anxiety, or psychosis, severe renal insufficiency, excessive concurrent use of alcohol

(c) Warnings: Administration of cycloserine should be discontinued or the dosage reduced
if the patient develops allergic dermatitis or symptoms of CNS toxicity

(d) The risk of convulsions is increased in chronic alcoholics.

c) Rationale for the use of cycloserine

The rationale for the use of DCS in pain derives from both animal studies as well as clinical experience with this drug. In addition to having antimicrobial properties, DCS is a partial agonist of the N-methyl D-aspartate receptor and has penetrance of the blood brain barrier. When DCS is administered either systemically or directly into the amygdala, it potentiates extinction and produces reduction in fear memory expression.[33, 61] This suggests that DCS might achieve a meaningful and long- lasting reduction in fear in anxiety disorders, and studies do show beneficial effects in a variety of anxiety disorders.[62-64] However, DCS did not produce an obvious reduction in fear in posttraumatic stress disorder.[65] We have shown that when DCS is administered orally (gavage), or in the medial prefrontal cortex, or the amygdala (with no effects seen in the spinal cord) it diminishes neuropathic pain behavior in a rodent model, with long-lasting after effects, and with enhancement of efficacy with repeat treatment. Perhaps more importantly when the DCS treated animals were tested using a place preference paradigm to assess the affective component of the neuropathic state, there was complete reversal of place

preference, suggesting that the emotional burden of the condition had been dramatically reduced or eliminated.[66] Interestingly in a patient with refractory orofacial pain transcranial direct current stimulation over hand motor area achieved pain reduction to 60% for at least 6 weeks, only when therapeutic stimulation was combined with DCS.[67] Additionally, at dosages of DCS that may reduce chronic pain (250 mg bid and lower) it exhibits minimal side effects. In studies of neurodegenerative, neurologic and psychiatric conditions,[62, 68] when DCS has been used at dosages of 10 mg to 200 mg/day for periods of up to 6 months, no reports of unexpected adverse effects have been noted. A Cochrane review report in people being treated for Alzheimer's disease[30] found no increase in withdrawals for adverse effects in subjects on DCS (dosages up to 200mg bid) compared to placebo.

d) Rationale for the dosage of cycloserine

The choice of dosage proposed in this study is based on the results of our previous pilot trial and preclinical data. From the animal studies our lab has done,[66] it appears that DCS may have an inverted U-shaped dose-response curve, i.e., at the highest doses there could be a diminution of efficacy. Because of that possibility, in our pilot trial we used a step-wise up-titration of DCS, starting at 50mg bid, going to 100mg bid and then 200mg bid, each for a 2 week period. What we observed was a maximum pain response at the end of the study at the highest dose, suggesting either that the highest dose was the most effective or that there was a time effect, or both. Regardless, the highest dose did not lessen the response, and therefore we feel confident that 200mg bid is an appropriate dose to demonstrate efficacy while providing a wide margin for safety.

e) Masking of cycloserine

The cycloserine will be manufactured by Keefer's Pharmacy (Arlington Heights, IL) as 200mg capsules, utilizing lactose USP as excipient and a size 0 hard gelatin opaque blue capsule (04.000, Capsugel®).

5.2. Placebo

A matching capsule containing lactose USP will be prepared by Keefer's Pharmacy. The appearance and properties of this capsule will be identical to that containing the active treatment and all labeling will be identical other than participant identifier.

5.3. Acetaminophen

Acetaminophen will be used as a rescue medication, up to 3000 mg/day. Tablets of 500 mg will be purchased in bulk and bottles of 100 given to subjects to be used as needed, up to 6 tablets/day. Bottles of rescue medication will be returned at each visit to allow drug accountability.

Acetaminophen has antipyretic and analgesic properties. It is generally safe and well-tolerated at doses up to 4 gm/day, though recent recommendations have tended to limit dosing to 3 gm/day, based on the fact that individuals are often unaware that they may be taking other medications that also contain acetaminophen, e.g., Tylenol #3, Norco®, Vicodin®. At doses significantly exceeding 4 gm/day, acetaminophen may result in hepatic damage and ultimately liver failure. Individuals using more than the equivalent of 2 beers or glasses of wine/day may have greater risk to acetaminophen toxicity. Acetaminophen has been associated with a number of other uncommon side effects, such as renal insufficiency, though causal relationship has not been demonstrated.

5.4. Participant Adherence and Accountability Procedures for the Study Product

Participants will be required to record all medication usage using the study eDiary. These data are immediately logged into the study server, which provides us routine overview of participants' participation and compliance, and further enhances retention of participants. If participants repeatedly miss entering these values, they are contacted by phone and instructed for compliance. Participants will be asked to return all unused study medication at each visit. Participants will be asked if they missed any days of study medication and if so, how and why. Participant will also be asked if they exceeded taking three capsules per day. The coordinator is responsible for conducting pill counts at each study visit.

5.5. Concomitant Medications/Treatments

Participants are asked to discontinue or washout of their current pain medications prior to and during the course of the study. The minimum washout period is 2 days (48 hours) for all prohibited medications that have an elimination half-life of less than 10 hours or at least 5 times the elimination half-life for those medications with longer half-lives. Participants will be allowed to continue their existing non-pain medications as long as they are not exclusionary for study enrollment. Common concomitant medications not permitted in this study are opioids, gabapentinoids, and NSAIDs. A complete list is included in the inclusion criteria. Participants may resume their prohibited medications once treatment is complete.

6 STUDY PROCEDURES/EVALUATIONS

6.1. Study Visit Procedures

- Medical history will be obtained through interview. All past medical history will be included.
- Medications history will be obtained through interview. Both prescription and over-the-counter medications from the previous two weeks to currently taking will be recorded.
- AEs and SAEs will be obtained at each study visit to assess participant safety. (See Section 9.1 Specification of Safety Parameters for further details)
- Physical examination will be obtained during the screening visit.
- Vital signs obtained by the study coordinator at the beginning of each visit include height (only at screening visit), weight (only at screening visit), BP, heart rate.
- Daily subject-reported outcomes: The study eDiary will be used to collect pain, mood, and medication use on a daily basis for the duration of the study.
- Blood samples will be collected from all participants at baseline visit. Urine samples will only be collected from WOCBP at the first dosing visit.
- Administration of PROs (See below for a comprehensive list)

6.2. Brain Imaging Procedures

- T1-MRI, DTI-MRI, resting state fMRI and ASL will be performed as described (Baliki M, 2012) (Mansour A, 2013) at baseline (Randomization), Week 12, and Early Withdrawal (if occurring before Week 12) with pulse and respiration rates obtained during the scans.
- All brain imaging is done on two 3.0 T Siemens Trio whole-body MRI scanner with echo-planar imaging (EPI) capability and using a 64 channels head-coil. Both scanners are dedicated for research and shared between 15 NIH funded research groups. The university has guaranteed us access to these magnets for the next 5 years. The magnets are managed by Radiology Department and are fully staffed and equipped. They are core human imaging facilities of NU. Their signal-to-noise ratio is excellent and they have produced very nice results over the last 5 years of operation, and >100 publications.
- We will acquire four different types of brain images:
  - Anatomical T1-MRI (high resolution anatomical magnetic resonance imaging): voxel size = 1 × 1 × 1 mm, repetition time = 2.3 s, echo time = 2.4 ms, flip angle = 9°, in-plane matrix resolution = 256 × 256; 176 slices, field of view = 256 mm.
  - Resting state fMRI (functional MRI): multi-slice T2*-weighted echo-planar images with repetition time = 555 ms, echo time = 22 ms, flip angle = 47°, number of volumes = 1110, slice thickness = 2 mm, voxel resolution = 2 x 2 x 2 (mm). The slices are covering the whole brain from the cerebellum to the vertex. A second resting state will also be performed if time allows.

  - DTI-MRI (diffusion tensor imaging): diffusion weighted images are acquired along 30 and 64 isotropic directions using b values of 700 and 2000 s × mm-2, respectively. Field of view=1044 x 1044, repetition time = 3.5 s, echo time = 92 ms, flig angle = 90. .
  - ASL (arterial spin labeling): The ASL sequence applies a pseudo-continuous label scheme to temporarily label or change the magnetic properties of the flowing blood spins. A “label” image with altered blood signal is acquired with a 3-dimensional readout method that facilitates removal of background tissue signal using appropriate radiofrequency pulses. A “control” image where the magnetic properties of blood spins are not altered is also acquired. Subtraction of the label image from the control image further removes non-perfusion related signal and generates a perfusion map that can be converted to physiological units (mL/100 g tissue/minute) based on published quantification models. Six alternating pairs of control and label images are acquired for signal averaging, which reduces noise.
- Brain imaging data analysis is done on free-share software including FSL, SPM, FreeSurfer, Caret, MRIcron as well as ad-hoc routines written in Matlab, C++, Pearl, Awk. The university maintains a user contract for Matlab and other software that we use routinely. We have a dedicated server for brain imaging data analysis. This is a 23 TByte data management system and with large number of processors, which runs on Linux. This server is our main brain imaging data storage and processing system. It is accessible through Internet, and all data processing is performed on it, mainly by remote access.

6.3. Laboratory Procedures/Evaluations

Baseline laboratory values will be collected for determination of inclusion/exclusion criteria and to assess safety and also will be entered into the study database. Repeat laboratory testing will only be done if there is a clinical basis for further testing. Blood and urine samples will be collected at the first study visit. Urine pregnancy tests will be processed before starting any study medication. Blood samples will be processed through Pathology Laboratory at NMH, 251 E. Huron St., Chicago, IL 60611. All results will be faxed to the study doctor for review before participants begin study medications. Samples will not be frozen nor stored for long-term or future use.

Screening laboratory evaluations will include complete blood count and differential, urine screen for pregnancy and illicit drug use, and a chemistry panel (16 tests that assess liver and kidney function and electrolyte balance.

6.4. Surveys and Questionnaires

Tools that we plan on using as part of the proposed research are appended and include the following:

Numerical Rating Scale (NRS)[69]: is an 11-point numerical rating scale used to measure pain intensity, where 0 corresponds to no pain and 10 indicates worst possible pain. The timeframe in question is concerned with the average pain experienced in the past 48 hours. This questionnaire has been extensively used across pain management studies.

Patient Global Assessment (PGA): A 5-point scale used to reflect the global impact of pain from the patient's perspective.

Patient Global Impression of Change (PGIC): A 7-point self-report measure that reflects a patient's belief about the efficacy of treatment by depicting a patient's rating of overall improvement.

McGill Pain Questionnaire - Short Form (sf-MPQ)[70]: is a well-validated pain measure, which permits separation of sensory and affective components of pain, as well as a total score. It also includes a numeric/descriptor scale, a visual analog pain scale (VAS), and a body map to localize the pain. This instrument is very well validated and often used in pharmacological research.[71]

Pain DETECT (PD-Q)[72, 73]: A 12-item self-report instrument that assesses neuropathic pain properties and was originally developed in a sample of 8,000 low back pain suffers in Germany. The PD-Q demonstrates high sensitivity (80%) and specificity (85%), and has since been validated in other clinical pain populations.

Positive and Negative Affect Schedule (PANAS)[74]: is a 20-item self-report instrument that measures positive and negative mood states. The PANAS was developed with a sample of undergraduate students and validated with adult populations. It is comprised of two mood scales, one measuring positive affect and the other measuring negative affect. Each item is rated on a 5-point scale ranging from 1 = very slightly or not at all to 5 = extremely to indicate the extent to which the respondent has felt this way in the indicated time frame.

Pain Catastrophizing Scale (PCS)[75]: The PCS is a 13-item instrument derived from definitions of catastrophizing described in the literature as well as items from the catastrophizing subscale of the CSQ. The PCS instructions ask participants to reflect on past painful experiences, and to indicate the degree to which they experienced each of 13 thoughts or feelings when experiencing pain, on 5-point scales with the end points (0) not at all and (4) all the time. The PCS yields a total score and three subscale scores assessing rumination, magnification and helplessness. The PCS has been shown to have adequate to excellent internal consistency (coefficient alphas: total PCS = .87, rumination = .87, magnification = .66, and helplessness = .78).

NIH PROMIS-57 Profile[79]: This 57-item questionnaire contains brief scales for

anxiety, depression, fatigue, pain interference, pain intensity, physical function, sleep disturbance, and the ability to participate in social roles. Each scale was created using PROMIS methodology and calibrated using item response theory.

The NRS pain scale data will comprise the elements of the primary endpoint for this study. The other instruments measure different attributes of pain, function and mood and will be used in the development of a model and clinical instrument to predict response to treatment (Aim 3). Participants will complete all the questionnaires in a quiet room by inputting responses directly into a computer-interfaced web-based system which will result in data being stored directly into the REDCap database. Schedule for when these questionnaires will be administered is included below in the study schedule.

Beck Depression Inventory (BDI): A 21-item, self-report rating inventory that measures characteristic attitudes and symptoms of depression (Beck, et al. 1961), including mood, pessimism, sense of failure, self-dissatisfaction, guilt, punishment, self-dislike, self-accusation, suicidal ideas, crying, irritability, social withdrawal, indecisiveness, body image change, work difficuly, insomnia, fatigability, loss of appetite, weight loss, somatic preoccupation, and loss of libido. (Beck & Steer, 1993: Beck, Steer & Garbing, 1988).

Multidimensional Assessment of Interoceptive Awareness (MAIA): A 32-item self-report measure of interoceptive body awareness that measures how aware someone is of his/her body and how well they can focus on bodily sensations (such as pain) or distract themselves from these sensations. Such a quality may also link to the propensity to respond or not respond to a placebo. The ability to control awareness or to just be more aware of one's body in general may also link to brain imaging data related to the functional connectivity of the insula and the mPFC.

Oswestry Disability Index (ODI): A 10-item, self-report questionnaire that has been validated specifically for measuring and quantifying degree of disability and estimating quality of life in those with back pain. (Fairbanks & Pynsent, 2000)

SF-12 Health Survey: An abbreviated 12-item measure of quality of life adapted from the widely utilized SF-36 Health Survey which relies upon patient self-reporting for routine monitoring and assessment of care outcomes in adult patients.

Chronic Pain Acceptance Questionnaire (CPAQ): A 20-item questionnaire measuring the effort participants put into either actively controlling their pain or passively accepting their pain.

Pain Anxiety Symptoms Scale (PASS-20): A 20-item questionnaire measuring pain-related fear, avoidance, and anxiety.

Pain Sensitivity Questionnaire (PSQ): A 17-item questionnaire assessing participants' sensitivity to imagined painful and non-painful stimuli.

Emotional Regulation Questionnaire (ERQ): A 10-item questionnaire measuring two kinds of strategies people use to control their positive and negative emotions.

Attentional Control Scale (ACS): A 20-item questionnaire assessing the voluntary control of attention during a variety of situations.

Emotional Attentional Control Scale (eACS): An 18-item questionnaire assessing the voluntary control of attention during emotionally demanding situations, which could include pain.

Five Facets of Mindfulness Questionnaire (FFMQ): A 39-item questionnaire measuring the five main components of mindfulness—observing, describing, acting with awareness, non-judging of inner experience, and non-reactivity to inner experience—as a skill set.

NEO Five Factor Inventory (NEO-FFI): A 60-item questionnaire measuring participants' scores on the personality dimensions of extraversion, agreeableness, conscientiousness, neuroticism, and openness.

Life Orientation Test-Revised (LOT-R): A 10-item questionnaire measuring dispositional optimism.

Loss Aversion Questionnaire (LAQ): A 20-item questionnaire measuring how sensitive participants are to a wide variety of potential "losses" in their lives.

NIH Toolbox: A comprehensive set of neuro-behavioral assessments that quickly assess cognitive, emotional, sensory, and motor functions. The various outcome measures can be used in reference to a normative dataset to identify baseline differences and patient profiles related to treatment success or failure. Additionally, longitudinal changes may be associated with treatment response and can be used in a predictive capacity.

7. ASSESSMENT OF SAFETY

7.1. Experimental Therapy

D-cycloserine has well described adverse effects that are largely based on drug levels (see Benefits/Risks section below). Participants will be monitored for evidence of hematologic, renal and liver function studies prior to entry, and a careful medical history with focus on depression and anxiety as well as concomitant medications will be taken. Individuals with renal insufficiency will not be enrolled. Other exclusion criteria are outlined above and will ensure that individuals at higher risk of DCS toxicity will not be enrolled.

7.2 Adverse Event Monitoring

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of subjects enrolled in the studies, as well as those who will enroll in future studies using similar agents. Adverse events are reported in a routine manner at scheduled times during a trial. Additionally, certain adverse events must be reported in an expedited manner to allow for optimal monitoring of patient safety and care.

All patients experiencing an adverse event, regardless of its relationship to study compound/drug, will be monitored until:

- the adverse event resolves or the symptoms or signs that constitute the adverse event return to baseline;
- any abnormal laboratory values have returned to baseline;
- there is a satisfactory explanation other than the study compound/drug for the changes observed; or
- death.

The reporting of adverse events, serious adverse events and unanticipated problems will be done according to the guidelines of the Northwestern University IRB and the FDA*. As of August 20, 2007, Northwestern University IRB requires filing of internal or external adverse events reports, or safety reports only if they have been determined by the Principal Investigator to contain a report of unanticipated problems involving risks to subjects or others. NU considers unanticipated problems, in general, to include any adverse event, incident, experience, or outcome that meets all of the following criteria: (1) Unexpected (in terms of nature, severity, or frequency) given: (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied. 1. The known or foreseeable risk of adverse events associated with the procedures involved in the research that are described in: a) the protocol-related documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed

consent document; and b) other relevant sources of information, such as product labeling and package inserts; or 2. The expected natural progression of any underlying disease, disorder, or condition of the subject(s) experiencing the adverse event and the subject's predisposing risk factor profile for the adverse event. (2) Related or possibly related to participation in the research (in this guidance document, 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 (3) Suggests that the research places subjects or others at a different or greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

NOTE:
The following events are considered a serious adverse event and would place subjects at a greater risk of harm:

- *results in death;*
- *is life-threatening (places the subject at immediate risk of death from the event as it occurred);*
- *results in inpatient hospitalization or prolongation of existing hospitalization;*
- *results in a persistent or significant disability/incapacity;*
- *results in a congenital anomaly/birth defect; or*
- *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 (examples of such events include allergic bronchospasm requiring intensive treatment in the 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).*

Unanticipated problems involving risks to subjects or others should be reported to the IRB within 10 working days (unanticipated deaths of subjects enrolled at NU or Affiliates need to be reported within 24 hours).

All adverse events deemed to be serious, both unexpected and expected, will be reported to the medical monitor.

Unanticipated problems involving risk to volunteers or others, serious adverse events related to participation in the study and all volunteer deaths related to participation in the study will be promptly reported by phone, by e-mail, or by facsimile to the FDA*.

The medical monitor is required to review all unanticipated problems involving risk to volunteers or others, serious adverse events and all volunteer deaths associated with the protocol and provide an unbiased written report of the event to the FDA and DOD. The medical monitor will comment on the outcomes of the event or problem and in the case of a serious adverse event or death comment on the relationship to participation in the study. The medical monitor will also indicate whether he/she concurs with the

details of the report provided by the study investigator. Reports for events determined by either the investigator or medical monitor to be possibly or definitely related to participation and reports of events resulting in death will be promptly forwarded to the FDA and DOD.

A summary table of adverse events collected will be submitted at least annually to the Northwestern IRB and to DOD.

7.2.1. Reporting of SAEs

SAEs that are unanticipated, serious, and possibly related to the study intervention will be reported to the Independent Monitor, IRB, and DOD in accordance with requirements.

- Anticipated or unrelated SAEs will be handled in a less urgent manner but will be reported to the Independent Monitor, IRB, DOD, and other oversight organizations in accordance with their requirements.

- Unexpected fatal or life-threatening AEs related to the intervention will be reported to the DOD Program Officer within 7 days.

7.3 Unblinding Procedures

Unblinding the study therapy may be necessary to ensure a subject's safety. In most cases, the unblinding will be part of managing an SAE, and will be reported with the SAE, however, in cases where unblinding was not associated with an SAE, such actions should be reported in a timely manner (e.g., notification of IRB within 24 hours by phone or fax, followed by a written narrative of the event within 48 hours.). Otherwise, unblinding will not be done until each phase of the study is complete (that is, when Aim 1 is finished running all enrolled participants, we can unblind the data).

7.4 Stopping Rules/Intervention Discontinuation

This study will be stopped prior to completion if: (1) the intervention is associated with adverse effects that call into question the safety of the intervention; (2) difficulty in study recruitment or retention will significantly impact the ability to evaluate the study endpoints; (3) any new information becomes available during the trial that necessitates stopping the trial; or (4) other situations occur that might warrant stopping the trial.

8. STATISTICAL CONSIDERATIONS

8.1. Sample size determination

The primary aim of this analysis is to determine whether DCS has a superior effect on pain ratings, as compared with the pill placebo control group, post-baseline. Data from our pilot trial indicate that we can expect an effect size of Cohen's *d* = 0.4 between groups. Thus, to achieve 80% power with α = 0.05 would require 98 subjects/arm. With a 20% dropout rate at 3 months (primary endpoint), we would require 122 participants/arm or 244 participants overall. Thus, we will plan to recruit a total of 244 participants into this study (122 into each group – treatment versus control). This would require randomizing 80 participants/year over 3 years which is similar to what we have accomplished recently. Figure 9 shows a plot of power to detect group differences at the endpoint (Time 4, 12 weeks) as a function of total sample size. As shown in the figure, if our retention rate is higher, then power will be even greater, exceeding 85% for total *N* = 244. If the true efficacy effect size is less than 0.4, this study may be underpowered to demonstrate this outcome, given the current sample size. However, effect sizes lower than 0.4 are not considered to be clinically effective, and so utilizing a larger population to allow statistical but not clinical significance is not warranted.

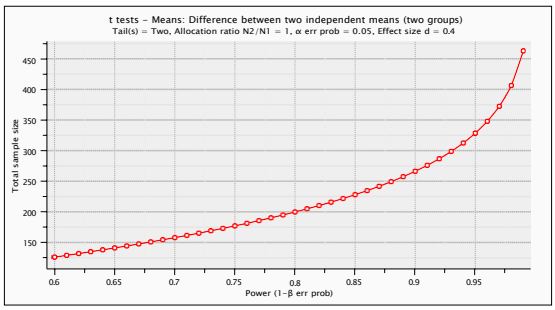


Fig. 9. Power vs N

One secondary aim is to identify brain biomarkers to allow prediction of response. For this analysis, our primary comparison is between categories of DCS response. Half of the data will be used for discovery, and the other half for validation. In the discovery phase, we will perform whole-brain contrasts (with proper correction for multiple comparisons) where we will have 50-60 subjects per group with 2 observations per subject contrasted to each other. This provides power >80% for detecting effects sizes of 0.50 or greater, assuming alpha = 0.01 (two-tailed). Our published whole-brain contrasts have been based on an average of n = 25 subjects per group. Comparison between groupings for behavioral outcomes should also be able to detect similar effect sizes. We have doubled the group sizes to ensure adequate power, as there are no data regarding the effects of DCS on CBP brain in comparison to CBP treated with placebo or healthy controls. We will also recruit a diverse population with comorbidities and likely high variability, and we plan on an aggressive brain-motion correction given recent reports of its large effect on network properties, and also because there are concerns that fMRI statistics have been inflated in the past (note: we will strictly use permutation testing to ensure statistics are precise). Only a minimal set of results will be tested for replication in the validation data.

For model building, our power analyses indicate that a sample size of 100 will be adequate, even after accounting for missing data and using assumptions based on our prior work. If we approach the analyses by classifying people into responders versus non-responders (50% expected in each group), statistical power would be adequate; assuming overall N = 100, an odds ratio of 2.0 as the effect size for a target predictor (which would be clinically significant) with an overlap of other predictors of R^2 = .09 (i.e., a medium-sized relationship with other predictors), and a two-tailed Type I error rate of .05, power would exceed 85%. Power would still be above 80% even assuming a 10% dropout rate (thus N = 90). These power analyses are based on a binary classification of the dependent variable, but we are also well familiar with methods that treat the dependent variable as continuous (e.g., mixed modeling, latent growth curve modeling), and these techniques will serve to increase statistical power if needed. Dr. Griffith is familiar with these approaches. We will examine receiver operating characteristic (ROC) curves to determine an optimum cutoff score. For the validation of the model, the algorithm derived from the analysis of the initial cohort will be applied to subsequent cohort. We will also evaluate the efficiency of the model and the a priori cutoff in terms of overall predictive accuracy, sensitivity, and specificity. For all analyses, cases with and without missing data will be compared, and multiple imputation strategies will be explored if assumptions are met.

8.2. Analysis plan for the primary endpoint: Pain NRS

Our first objective is to determine the efficacy of DCS compared to placebo to reduce pain in people with chronic low back pain. Thus, the primary outcome of this trial is the treatment's effect (DCS) on the average of ecological momentary assessment-based pain ratings collected during the 12th week of the study relative to placebo estimated using intention-to-treat (ITT). This will be performed for those who were randomized before March 1st, 2022.

The average of ecological momentary assessment-based pain ratings for baseline will be calculated as the average pain rating in the week preceding randomization—all pain ratings during this period will be used. Since participants completed at least four rating during this period, there are no missing data at baseline. The average of ecological momentary assessment-based pain ratings for the 12th week will be calculated as the average pain rating during week 12—all pain ratings during this period will be used. Only participants who did not rate during this period will be treated as missing. Missing data will be handled in accordance with the missing data mechanism(s). Participants were categorized into six categories based on the mechanism of missingness:

1. Withdrew consent
2. Lack of efficacy
3. Loss to follow-up
4. Adverse events
5. Noncompliance

6. Study ended early

We will impute missing values based on these categories.[1] Our imputation models will use baseline pain, age, and sex, in addition to previous and 12-week visit pain ratings. Patients in categories (1), (3), (5), and (6) will be treated as missing at random and rely on imputation models derived from the groups to which they were randomized. In contrast, patients in categories (2) and (4) will rely on a "jump to reference" assumption, for which we will rely on imputations using a model derived from the placebo group.[2] We will employ Multivariate Imputation by Chained Equations (MICE) to account for the uncertainty in the imputation model.[3] Importantly, imputation will be performed separately for each group to capture interaction effects appropriately, should they be present (e.g., different mechanisms of missingness for DCS and placebo).[4]

Our primary outcome will be estimated using ordinary least squares. We will evaluate the adjusted effect of treatment on post-intervention pain ratings in accordance with ITT.[5,6] We will include pre-intervention pain as a covariate, which has prognostic value and thus often improves precision.[5,7] The model for our primary outcome will take the form,

$$y_i = \beta_0 + \beta_1 TX_i + \beta_2 Pre_i + \epsilon_i,$$

where y_i is the post-intervention pain for patient *i* (average of 0–10 NRS pain ratings over the 12th week); Pre_i is the pre-intervention pain score for patient *i* (average of 0–10 NRS pain ratings over one week at baseline); TX_i is dummy-coded 1 if patient *i* was randomized to DCS and 0 if they were randomized the placebo group. We will assess model fit and modify the model as necessary to ensure adequate fit (e.g., by transforming pain ratings to, for example, the log scale [7]); all modifications will be transparently reported. This model will be fit using ordinary least squares. β_1 is the parameter of interest.

Our outcome of interest is the adjusted effect of DCS relative to placebo on pain at 12 weeks. For inferences, we will rely on an estimation approach;[8-10] we will present all compatibility (or confidence) intervals (CI) (0–100%) as a consonance or confidence function generated using concurve.[11] For readers, we will also present (a) a 95% CI of the point estimate alongside (b) a *P*-value based on the sharp point null hypothesis of zero difference between DCS and placebo by performing a randomization test, wherein we will re-randomize patients consistently with the block randomization scheme.

8.3. Sensitivity analyses for primary outcome

Sensitivity Analyses for the Primary Outcome
We will perform several sensitivity analyses for the primary outcome, each of which is detailed below.

1. **ITT (all participants)**: Perform the same analysis as the primary outcome, but we will

include all participants rather than just those randomized before March 1st, 2022.

2. **As-treated analysis (all participants)**: Perform the same analysis as (1), but instead of ITT, use the as-treated group assignments.
3. **Per-protocol (randomized before March 1st, 2022)**: Using an identical model to the one used for our primary outcome, we will perform the same analysis but per-protocol. That is, we will only include participants randomized before March 1st, 2022, and participants for whom we do not have data at 12 weeks or who have had major protocol deviations will be dropped from the analysis.
4. **Per-protocol (all participants)**: We will perform the same analysis as in (3) but with all participants. Group assignments used in this analysis will be the group to which they were expected to be randomized (cf. the as-treated analysis).
5. **MNAR**: We will consider a range of missing data mechanisms by assuming that those for whom we do not have data improved by different amounts (NRS pain decreasing by 0.5–4 in intervals of 0.5, relative to the MAR-based imputed value, with a floor of 0) or worsening by different amounts (NRS pain increasing by 0.5–4 in intervals of 0.5, relative to the MAR-based imputed value, with a ceiling of 10). We will apply these to each group separately, such that we will look at all combinations in DCS vs. all combinations in placebo ((8 improving + 8 worsening)^2 = 16^2 = 256 combinations); e.g., if those in DCS worsened by 4 while those in the placebo improved by 2. This is conceptually equivalent to a "tipping point" analysis.
6. **Covariates**: We will adjust the primary analysis for additional covariates that we do not suspect would have prognostic value, including age, sex, race, ethnicity, socioeconomic status, and pain duration.
7. **Cumulative distribution functions**: We will plot the cumulative distribution functions of patient outcomes, adjusted for pre-intervention pain.

8.4. Secondary Outcomes.

As a secondary outcome, we will investigate the persistence of the effect of DCS 12 weeks after the DCS patients were switched to placebo—i.e., 24 weeks after the start of the trial. That is, we will estimate the drug's longitudinal effect at 24 weeks. The model will be specified identically to that for the primary outcome (efficacy at 12 weeks).

8.5. Exploratory Outcomes.

Our exploratory outcomes will include (1) a longitudinal analysis of the data that includes all time points and treats time continuously; (2) the effects of DCS on other patient outcomes, including their perception of the impact of their condition, pain characteristics, affective and personality indices; (3) the role of the corticolimbic and hippocampal circuitry in DCS's treatment effects; (4) assess DCS safety in a chronic low back pain population.

1. *Longitudinal treatment effect*

Our primary outcome solely focuses on the treatment effect in the final week of DCS administration. However, pain relief before 12 weeks and the persistence of pain relief are both clinically important outcomes. Here, we will build on this outcome and examine the treatment effect across time, from week 1 through week 24. To do so, we will fit a generalized additive model using all pain rating data. Smooths will be created as a function of time since

randomization, which will vary by group. We will fit this model in two ways: (1) Using random effects, such that each individual's time course is modeled. (2) Like a generalized estimating equation, using an error covariance structure to account for repeated measures. We will inspect the residuals; if autocorrelation is present, we will adjust the error covariance structure accordingly. The final outcome of this model will be the contrast between the DCS group's smooth and the placebo group's smooth—the average treatment effect across time.

2. *Questionnaires*

We collected several questionnaires to assess the effect of the intervention on complementary aspects of patients' lives. Specifically, the following questionnaires were collected:

- Patient Global Assessment
- Patient Global Impression of Change
- McGill Pain Questionnaire (obtained at Screening, Week 12, and Week 24)
- painDETECT instrument (obtained at Screening, Week 12, and Week 24)
- Beck Depression Inventory – Second version (obtained at Screening, Week 12, and Week 24)
- Positive and Negative Affect Schedule (obtained at Screening, Week 12, and Week 24)
- Pain Catastrophizing Scale (obtained at Screening, Week 12, and Week 24)
- Multidimensional Assessment of Interoceptive Awareness (obtained at Screening, Week 12, and Week
- Oswestry Disability Index (obtained at Screening, Week 12, and Week 24)
- SF-12 Health Survey (obtained at Screening, Week 12, and Week 24)
- Chronic Pain Acceptance Questionnaire (obtained at Screening and Week 24)
- Pain Anxiety Symptoms Scale (obtained at Screening and Week 24)
- Pain Sensitivity Questionnaire (obtained at Screening and Week 24)
- Emotional Regulation Questionnaire (obtained at Screening and Week 24)
- Attentional Control Scale (obtained at Screening and Week 24)
- Emotional Attentional Control Scale (obtained at Screening and Week 24)
- Five Facet Mindfulness Questionnaire (obtained at Screening and Week 24)
- Neuroticism-Extraversion-Openness (NEO) Five Factor Inventory (obtained at Screening and Week 24)
- Life Orientation Test (Revised) (obtained at Screening and Week 24)
- Loss Aversion Questionnaire (obtained at Screening and Week 24)

The effect of the intervention on these questionnaires will be modeled similarly to our primary outcome but with a few exceptions. First, for questionnaires with few outcomes, we will rely on ordinal regression. Second, for outcomes and questionnaires collected only at the end of the study, baseline will be left out of the model. Finally, for outcomes and questionnaires collected multiple times, we will include a factor for time in the model.

3. *Functional Neuroimaging*

Functional magnetic resonance imaging (fMRI) data were collected at randomization and 12 weeks to provide mechanistic insight into the drug's treatment effects. With these data, we will investigate the activity (ALFF) and functional connectivity of five pre-specified brain regions:

nucleus accumbens (NAc), medial prefrontal cortex (mPFC), hippocampus, amygdala, and posterior cingulate. Several of these regions follow from previous work from our group.[12,13] Based on these areas, we will fit the following models:

- The effect of DCS on mPFC, Hippocampus, NAc, and amygdala activity will be specified similarly to the ANCOVA used in our primary outcome model.
- The effect of DCS on mPFC, Hippocampus, NAc, and amygdala connectivity will be specified similarly to the ANCOVA used in our primary outcome model.
- Assess how mPFC, hippocampus, NAc, or amygdala activity mediates the effect of DCS.
- Assess how mPFC, hippocampus, NAc, or amygdala connectivity mediates the effect of DCS.
- Assess how the baseline activities moderate DCS's treatment effect.
- Assess how the baseline functional connectivities moderate DCS's treatment effect.
- A mediation model that includes the moderation effects, wherein we will assess if the activities/functional connectivities at 12 weeks explain post-intervention pain.

4. *Adverse Events*

We will summarize safety endpoints common across clinical trials, including adverse effects, laboratory test results, and vital signs. All safety data will be presented in individual subject listings by group and subject, ensuring transparency and completeness of the safety data.

Descriptive statistics will be used to analyze all safety variables: continuous variables will be summarized by N, mean, median, standard deviation, and minimum and maximum values. Categorical variables will be presented in frequency tables with counts and corresponding percentages to provide a clear and comprehensive summary of the safety data.

The adverse effect (AE) listing will include:

- System organ class
- Preferred term
- Trial treatment at the onset of the event
- Event duration
- Resolution
- Severity
- Seriousness
- Relationship to Study Procedures
- Relationship to Study Drug

We will create a summary table to describe adverse events (AEs) overall and by group (DCS/Placebo). The table will include the frequency of events and the number and percentage of subjects experiencing these events by system and term, sorted in descending order of total frequency. Additionally, all AEs will be tabulated by severity in the same manner. This approach will provide a detailed overview of the safety profile of the intervention and enable us to identify any patterns or trends in the occurrence of AEs.

9. DATA HANDLING AND RECORD KEEPING

The investigators are responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. The investigators will maintain adequate case histories of study participants, including accurate CRFs, and source documentation.

9.1. Data Management Responsibilities

Data collection and accurate documentation are the responsibility of the study staff under the supervision of the investigator. All source documents and laboratory reports must be reviewed by the study team and data entry staff, who will ensure that they are accurate and complete. UPs and AEs must be reviewed by the investigator or designee.

9.2. Data Capture Methods

We plan to use REDCap (*http://www.project-redcap.org/*) as our data management tool. Participant-entered data will be collected directly into the REDCap application as source documents and subsequently printed out for participant files. PROs, will not contain any PHI and will be stored in participant's folder. Clinical (e.g., medical history and physical examination, pain evaluations) and functional (e.g., brain phenotype) status assessments will be entered on paper as source documentation and subsequently

entered into REDCap as confirmation of a participant's passing or failing inclusion/exclusion criteria, Other data collected by study staff pertaining to the study visit will be entered on paper and subsequently entered into REDCap.

9.3. Types of Data

Our research staff will be collecting multiple types of data pertaining to the participants, such as those dealing with safety, imaging, and behavioral and medical participant domains. The safety of the participant will be initially assessed through obtaining a thorough history and assuring that participants meet all the criteria of the inclusion/exclusion checklist, which reviews comorbid illnesses and contraindicated medications. Safety data will continue to be collected at each contact with participants and recorded in the source documents. There will not be a separate safety database. Imaging data will be collected during each of the scanning visits and will be collected based on well-documented protocols. Behavioral data will be provided through selected patient PROs that assess various aspects of pain and overall quality of life. Medical data will be collected primarily during physical examinations and from a baseline blood draw, in addition to follow-up visits throughout the study. These measurements include BP, heart rate, chemistry panel, CBC, drug urine screening, and urine pregnancy screening for women of child bearing potential.

9.4. Schedule and Content of Reports

In order to monitor the progress of the study and to provide quality control, a number of reports will be regularly generated throughout the course of the study. The clinical investigator will have a weekly meeting with all study staff to review the status of the study and address issues that have arisen during the week. At this meeting, a recruitment and retention report will be presented and discussed. Dates of important summary reports (e.g., annual IND report, report to clinical committee, IRB annual renewal, etc.) will be highlighted. In general, a draft of any report to be circulated outside the study team will be prepared at least one week prior to the deadline for the report needing to be sent in order to provide adequate time for review and discussion. It is anticipated that there will be a data review regarding safety done at least quarterly or more often as requested by the study oversight committee. Outcome data will not be reviewed until after database lock, though the integrity and completeness of data will be assessed on a quarterly basis as part of quality assurance activities. The database will not be locked until all data fields have been checked and any remaining discrepancies dealt with. Once the database has been locked, no further changes will be made and the randomization code can be provided to the study statistician.

9.5. Study Records Retention

Study records will be maintained for at least five years from the date that the grant FFR is submitted to the NIH. No records will be destroyed for up to 3 years after the study has completed. There is no anticipation of a marketing application.

9.6. Protocol Deviations

A protocol deviation is any noncompliance with the clinical study protocol or Good Clinical Practice requirements. The noncompliance may be on the part of the subject, the investigator, or study staff. As a result of deviations, corrective actions will be developed by the study staff and implemented promptly.

These practices are consistent with investigator and sponsor obligations in ICH E6:

- Compliance with Protocol, Sections 4.5.1, 4.5.2, 4.5.3, and 4.5.4.
- Quality Assurance and Quality Control, Section 5.1.1
- Noncompliance, Sections 5.20.1 and 5.20.2.

All serious and significant deviations from the protocol will be documented in the study participant source documents and promptly reported to NIDCR and the local IRB, according to their requirements.

9.7. Access to Source Documents

Study staff will maintain appropriate medical and research records for this study, in compliance with ICH E6, Section 4.9 and regulatory and institutional requirements for the protection of confidentiality of participants. All source documents pertaining to an individual participant is compiled into a participant folder. Each participant folder includes, but is not limited to, records on personal contact information, demographic information, physical exam, vital signs, personal health history, researcher comments from each visit, PROs, concomitant medication logs, study medication logs, indications of any AEs experienced, clinical laboratory results, and ICFs. PROs will be collected directly into REDCap (REDCap is the source document), but will be printed and maintained with the participant folder. All folders are kept in a locked cabinet, located in a locked room away from all participant activity. Only study coordinators have access to the locked room and cabinet. These paper charts are also kept separate from any hospital records acquired through NMH or the NMFF. Study staff will permit authorized representatives of NIDCR, the NU IRB and defined regulatory agencies to examine (and when required by applicable law, to copy) research records for the purposes of quality assurance reviews, audits, and evaluation of the study safety, progress and data validity.

All brain imaging data are maintained on a secure server, stored under lock at the NMH hospital server banks. Access to these data are password protected, and all brain scan data are anonymized and coded prior to being available on the server. Only NUCATS will maintain the lock for the specific subject-to-data correspondence, and this information is not anticipated to be used, except in some unusual emergency situation (such an event has not arisen in the past >15 years).

10. ETHICS/PROTECTION OF HUMAN PARTICIPANTS

The investigator will ensure that this study is conducted in full conformity with the principles set forth in The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Participants of Research, as drafted by the US National Commission for the Protection of Human Participants of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR Part 46 and/or the ICH E6.

10.1. Institutional Review Board

The protocol, ICF(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the ICF 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 in the study.

10.2. Informed Consent Process

Informed consent is a process that is initiated prior to the individual agreeing to participate in the study and continues throughout study participation. Extensive discussion of risks and possible benefits of study participation will be provided to participants and their families, if applicable. A consent form describing in detail the study procedures and risks will be given to the subject. Consent forms will be IRB-approved, and the participant is required to read and review the document or have the document read to him or her. The investigator or designee will explain the research study to the participant and answer any questions that may arise. The participant will sign the informed consent document prior to any study-related assessments or procedures. Participants will be given the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. They may withdraw consent at any time throughout the course of the study. A copy of the signed informed consent document will be given to participants for their records. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their clinical care will not be adversely affected if they decline to participate in this study.

The consent process will be documented in the participant folder. (See Appendix B for an example of the ICF)

10.3. Participant Confidentiality

We are committed to respect participant privacy and to keep personal information confidential. When choosing to take part in this study, participants are giving us the permission to use their PHI that includes health information in their medical records and information that can identify them. For example, PHI may include name, address, phone number or social security number. Health information that we may collect and use for this research includes:

- All information in a medical record
- Results of physical examinations
- Medical history including back pain history and family history of back pain
- Lab tests, or certain health information indicating or relating to a particular condition as well as information collected by cellphone apps and PROs
- Records about study medication or drugs
- Records about MRI scans
- Substance abuse information: current recreational drug use or history of alcohol or drug abuse

The following groups of people may give the researchers information about research participants: All current and previous health care providers, including but not limited to the NMFF, NMPG, NMH. Once we have the health information listed above, we may share some of this information with the following people. Please note that any research information shared with people outside of NU and its clinical partners (or affiliates) will not contain subject's name, address, telephone or social security number or any other direct personal identifier unless disclosure of the direct identifier is required by law [except that such information may be viewed by the Study sponsor and its partners or contractors at the PI's office]

- Authorized members of the NU workforce, who may need to see information, such as administrative staff members from the Office for Research, Office for Research Integrity and members of the Institutional Review Board (a committee which is responsible for the ethical oversight of the study),
- Clinical affiliates, including but not limited the NMFF, NMH, and NMPG. Individuals' participation in this clinical trial will be tracked in an electronic database and may be seen by investigators running other trials and by other healthcare providers having access to this database.
- Other University research centers and University contractors who are also working on the study,
- Study monitors and auditors who make sure that the study is being done properly,
- Government agencies and public health authorities, such as the FDA and the DHHS.

The results of this study may also be used for teaching, publications, or presentation at scientific meetings. However, the individual's name and personal information will not be used.

All imaging data and behavioral data will be uploaded to the Northwestern University Research Image Processing System (NURIPS), an online collaborative research environment for securely storing, managing, analyzing and sharing de-identified medical imaging, associated data (e.g. behavioral), and results from advanced customizable processing pipelines. NURIPS is supported by both NUIT and FSM-IT and takes advantage of the NU high performance computing cluster, Quest. NURIPS is a secure environment that supports the latest NU policy and procedures for encryption of data

during transit and rest, provides granular project level access controls with varying permissions based on user groups, and allows non-NU collaborators access once they obtain an affiliate NetID. All data are backed up and have restore points that go back for 30 days. Users have access to common data analysis pipelines and the opportunity to create and share their own pipelines

10.4. Future Use of Stored Specimens and Other Identifiable Data

We foresee that the brain imaging data and related PROs may be used in future studies, either by us or when we make this data available on OpenPain for use by other researchers (Apkarian, 2014). In all such cases all data will be fully anonymized prior to their availability for future use by investigators outside of Apkarian's lab.

10.5. Study Oversight

In addition to the PI's responsibility for oversight, study oversight will be under the direction of an Independent Safety Monitor (ISM), who has expertise in clinical trials. The ISM is independent of the study and will be available in real time to review and recommend appropriate action regarding adverse events and other safety issues. We anticipate safety concerns to be relatively limited due to the fact that (1) the safety profiles of both study medications have been well described over long periods of time, (2) the doses that are being used are at the lower end of the therapeutic range for d-cycloserine, (3) treatment is for a limited period of time (24) and (4) careful monitoring of safety will be undertaken by the study team with regular oversight by the clinical investigator and the principal investigator.

10.6. Risks and Benefits

Risks

After screening, all participants for these studies will be asked to discontinue their current medications that are being used to manage their CBP to allow for the assessment of their baseline pain status. Individuals not on medical management can qualify if their pain is of sufficient magnitude at the baseline visit. All participants will be informed of the possibility that they may be treated with placebo. They therefore must be willing and able to be off active medication for their pain for up to 12 weeks. Rescue medication in the form of acetaminophen 500 mg up to 3000 mg/per day will be allowed and available to all participants. Participants will be free to discontinue from the study if they are not able to deal with their pain.

MR scans

The MR environment is hazardous and only people properly trained regarding safety procedures are allowed. There is risk of claustrophobia and anxiety involved with the

fMRI scanning procedure; subjects will be queried about any concerns they may have prior to testing. Subjects must consent to tolerate being in the scanner, which involves the potentially uncomfortable experience of lying in a relatively confined space with minimal head and body movements for up to 30 minutes at a time. In this time period, their back pain may become worse. Personnel continuously query the subject in the scanner as to their level of pain and will discontinue the scan and relieve the subject from the discomfort at any time requested. Our routine procedure is to give subjects multiple breaks from scanning, which minimizes fatigue and decreases anxiety, resulting in high-quality data. Collectively, it takes about 50 minutes for all scans to be conducted. It is possible that the anatomical scans may uncover a brain abnormality of which the participant was not aware. In such cases, the participant is removed from the study and given appropriate medical advice (TJS). The likelihood of this happening is low (<1%). Over the last 5 years of doing brain scans in various chronic pain patient populations, the main limiting factor has been claustrophobia. Although we query subjects on this routinely, participants often are surprised at how anxious they feel in the scanner and simply cannot tolerate it. Otherwise, chronic pain patients are usually enthusiastic in participating in these trials.

Medications

D-cycloserine

D-cycloserine is currently approved by the FDA to treat tuberculosis and is an effective antimicrobial for a wide range of bacteria. It is known in some people to cause dizziness and drowsiness and has been associated with the development of seizures, particularly in association with alcoholic intake. It may also lead to and/or aggravate depression and suicidal ideation. People taking D-cycloserine should not drink alcohol and should understand how this drug affects them prior to driving or using machinery. Other side effects associated with D-cycloserine therapy are consistent with a central mechanism of action and include anxiety, confusion, increased irritability, increased restlessness, mental depression, muscle twitching or trembling, nervousness, nightmares, other mood or mental changes, and speech problems. Less commonly reported are seizures, numbness, tingling, burning pain, or weakness in the hands or feet and skin rash.

Administration is contraindicated in patients with hypersensitivity to cycloserine. Caution is advised when prescribing cycloserine for patients with epilepsy, depression, severe anxiety, or psychosis, severe renal insufficiency and excessive concurrent use of alcohol. These individuals will be excluded from enrollment into this study.

The toxicity of cycloserine is closely related to excessive blood levels (above 30ug/mL), as determined by high dosage or inadequate renal function. Toxicity and the most serious side effects have been reported at doses above 500 mg/day or in people with renal insufficiency. Drug interactions with cycloserine have also been described. Concurrent administration of ethionamide has been reported to potentiate neurotoxic

side effects. Alcohol increases the possibility and risk of epileptic episodes. Concurrent administration of isoniazid may result in increased incidence of CNS effects, such as dizziness or drowsiness. Cycloserine inhibits the hepatic metabolism of phenytoin and may increase risk of epileptic seizures.

The effects of cycloserine with regard to causing or exacerbating psychiatric disorders has not been well studied. In cases using cycloserine at high doses to treat tuberculosis (>500 mg/day), there have been reports of seizures and psychiatric disorders. The effects of cycloserine on individuals taking anti-psychotic medications has been studied by Goff et al (NCT00000371; Psychopharmacology (Berl). 2005 Apr;179(1):144-50. Epub 2004Oct 21) who found no differences compared to placebo in 55 schizophrenic patients studied. Based on a review of the literature available, the only drugs used to treat psychiatric disorders which are specifically identified as potentially having an interaction with cycloserine are buproprion and fluphenazine; thus, these drugs will be exclusionary for enrollment into this study.

Acetaminophen

Acetaminophen will be employed as rescue medication. Usage will be limited to < 3.0 gm/day and individuals will be cautioned against the use of alcoholic beverages, which are not to be used while in the study. Screening laboratory values for liver function tests will have to be within expected limits for study entry to prevent individuals with underlying liver disease to be included in the study. Every effort will be made to ascertain all the concomitant medications participants will be taking so that if acetaminophen is included in their daily treatment, appropriate reductions in maximum acetaminophen to be used as rescue medication can be made. Although acetaminophen has been implicated as a possible cause of renal insufficiency, use in these studies will be short-term and individuals with pre-existing renal insufficiency will be excluded.

Questionnaire Risks

Although the majority of participants will find the questionnaires to be harmless, mundane, or possibly interesting, there is always a possibility that some of the questions may be sensitive in nature and may make them uncomfortable (particularly those asking about mood or personality). In the past, we have added an option into our REDCap database that allows participants to skip questions they don't feel comfortable answering, so they will never be forced to answer something they do not want to. We have found that most people still answer all questions fully despite having this optional button and that most participants have no missing data. Therefore, we will continue to use this option in the proposed study.

Because we are measuring depression, anxiety, and other mental health constructs in these questionnaires, Dr. Griffith, a licensed clinical psychologist, will be available to consult about any concerns that patients have about their mental health, including

referrals to appropriate providers. All participants will be given a list of referral sources in case they wish to seek mental healthcare, and participants will be invited to contact the research team with questions about getting connected with appropriate healthcare. Additionally, the clinical research managers are trained in suicide prevention techniques should any immediate need arise during a study visit or follow-up.

Phlebotomy risk

The possibility exists for pain, ecchymoses and rarely localized infection as a consequence of phlebotomy. These will be minimized by careful aseptic technique and involvement of experienced phlebotomists.

Emergency care

If participants need emergency medical care, they will be instructed to go to the closest emergency room and inform the healthcare provider that they are enrolled in this study. If the need is not emergent, they will be asked to come to the clinic to be seen by Dr. Schnitzer, the PI. Participants will be responsible for the cost of any emergency or non-emergency medical care that may arise during or as a consequence of their involvement in this study.

Protections against Risk

General
The primary protection will be the enrollment only of eligible subjects, i.e., subjects meeting all inclusion and exclusion criteria. Individuals having clearly identified causes of low back pain will be carefully excluded. In addition, individuals who cannot tolerate the confinement of an MRI scanner and those who may not be able to be scanned because of implanted metal devices will be excluded. Following treatment administration, safety monitoring for non-serious and serious adverse events is planned at each study visit until the completion of the trials. All adverse events will be collected and reviewed by the clinical lead investigator and principal investigator on a monthly basis. A chronological log of all SAE's will be maintained and reviewed by TJS every month, as well. Privacy and confidentiality will be maintained by the use of allocation numbers throughout the study, with the master study list (excluding group membership codes) being maintained by the PI with availability limited to those study personnel who have a documented need for this information.

Protection against DCS risk
Patients will be monitored for evidence of hematologic, renal and liver function studies prior to entry, and a careful medical history with focus on depression and anxiety as well as concomitant medications will be taken. Individuals with renal insufficiency will not be enrolled. Other exclusion criteria are outlined above and will ensure that individuals at higher risk of DCS toxicity will not be enrolled.

Independent Research Monitor

As this study will result in more than minimal risk, an independent research monitor has been identified (Dr. Elliot Roth). At a minimum, the research monitor: may discuss the research protocol with the investigators, interview human subjects, consult with others outside of the study about the research; shall have the authority to stop a research protocol in progress, remove individual human subjects from a research protocol, and take whatever steps are necessary to protect the safety and well-being of human subjects until the IRB can assess the monitor's report; shall have the responsibility to promptly report their observations and findings to the IRB or other designated official and the HRPO. He will be actively involved in following participants' safety, reviewing all SAEs and being present at 6-monthly safety and protocol review meetings. He is experienced in this role having been the safety monitor for our previously funded clinical trials.

Benefits

The participants in this study who receive DCS treatment may benefit from pain relief during and after treatment. Many of the participants who receive placebo treatment are also expected to experience pain relief. Identification of patients who may be expected to respond to DCS or placebo would allow for a safer and more cost-effective approach to pain management while allowing for more targeted therapies to non-responders. This knowledge would also permit more efficient and productive clinical trials of new agents being tested for analgesic efficacy in CBP populations by allowing for the selective removal of placebo responders to discern true treatment effects. All participants will be carefully monitored.

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